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NEWS	3	Feb 1	Addition of Machine-Translated Abstracts to CAplus
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NEWS	11	Mar 20	INPADOC: PRODUCER WARNING ABOUT DATA DELAYS
NEWS	12	Mar 22	NEW FEATURES IN INPADOC - RANGE SEARCHING AND NEW SDI/UPDATE SEARCH FIELD
NEWS	13	May 1	Beilstein Abstracts on STN - FILE BABS
NEWS	14	May 1	RN CROSSOVER AND ANSWER SIZE LIMITS INCREASED
NEWS	15	May 1	AIDSLINE has been reloaded
NEWS	16	May 1	Searching Y2-K compliant Patent Numbers

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FILE 'MEDLINE' ENTERED AT 14:42:02 ON 02 MAY 2000

FILE 'BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000
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=> s cyclase and CRE

BEST AVAILABLE COPY

09/[illegible]046

rhythmic melatonin signal.

L5 ANSWER 4 OF 15 MEDLINE
ACCESSION NUMBER: 1999021198 MEDLINE
DOCUMENT NUMBER: 99021198
TITLE: Inducible cAMP early repressor ICER down-regulation of **CREB** gene expression in Sertoli cells.
AUTHOR: Walker W H; Daniel P B; Habener J F
CORPORATE SOURCE: Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston 02114, USA.
CONTRACT NUMBER: DK25532 (NIDDK)
SOURCE: MOLECULAR AND CELLULAR ENDOCRINOLOGY, (1998 Aug 25) 143 (1-2) 167-78.
Journal code: E69. ISSN: 0303-7207.
PUB. COUNTRY: Ireland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY WEEK: 19990301

AB The cAMP response element binding protein (**CREB**) and the cAMP-responsive element modulator (CREM) are cyclically expressed in the seminiferous tubules during spermatogenesis. In the somatic Sertoli cells, which are the major supporters of germ cell development in the seminiferous tubules, the expression of **CREB** is cyclical and appears to be regulated by the levels of cAMP produced in response to the pituitary derived follicle-stimulating hormone FSH. Cyclic AMP response elements (CREs) located in the promoter of the **CREB** gene were shown earlier to be implicated in an autopositive **feedback loop** that up-regulates the expression of **CREB**. Here we show that in Sertoli cells FSH-mediated induction of the CREM repressor isoform, ICER (inducible cAMP early repressor) is correlated with the inhibition and delay of **CREB** gene expression in the seminiferous tubules. ICER binds to the two CREs located in the promoter of the **CREB** gene and in transient transfection assays of Sertoli cells, ICER expression vectors down-regulate transcription of a reporter gene driven by the **CREB** gene promoter. In addition, analyses of ICER and **CREB** gene expression in isolated segments of rat seminiferous tubules reveals stage-specific and cycle-dependent expression of ICER. The periods of enhanced expression of ICER correspond to the stages of spermatogenesis with the lowest levels of **CREB** expression. We suggest that the expression of ICER in Sertoli cells may contribute to the periodic repression of **CREB** gene expression during the repeated 12-day cycles of spermatogenesis, and may be required to reset the levels of activator **CREB** prior to the initiation of

SYNERGISM COOPERATIVE?
CO TRANSFECTION?
REPRESS?
AMPLIF?

SCREEN, ASSAY, LIGAND
TRANSCRIPTION
[illegible] FEED BACK

L1 128 CYCLASE AND CRE

=> s (feedback loop)

L2 2756 (FEEDBACK LOOP)

=> s l1 and l2

L3 1 L1 AND L2

=> d ibib abs

L3 ANSWER 1 OF 1 MEDLINE
ACCESSION NUMBER: 97381623 MEDLINE
DOCUMENT NUMBER: 97381623
TITLE: Coupling signalling pathways to transcriptional control: nuclear factors responsive to cAMP.
AUTHOR: Tamai K T; Monaco L; Nantel F; Zazopoulos E; Sassone-Corsi P
CORPORATE SOURCE: Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, Strasbourg, France.
SOURCE: RECENT PROGRESS IN HORMONE RESEARCH, (1997) 52 121-39; discussion 139-40. Ref: 93
Journal code: R1D. ISSN: 0079-9963.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
ENTRY MONTH: 199710

AB Several endocrine and neuronal functions are governed by the cAMP-dependent signalling pathway. In eukaryotes, transcriptional regulation upon stimulation of the adenylyl **cyclase** signalling pathway is mediated by a family of cAMP-responsive nuclear factors. This family consists of a large number of members that may act as activators or repressors. These factors contain the basic domain/ leucine zipper motifs and bind as dimers to cAMP-response elements (**CRE**). The function of **CRE**-binding proteins (CREBs) is modulated by phosphorylation by several kinases. Direct activation of gene expression by CREB requires phosphorylation by the cAMP-dependent protein kinase A to the serine-133 residue. Among the repressors, ICER (Inducible cAMP Early Repressor) deserves special mention. ICER is generated from an alternative CREM promoter and constitutes the only inducible cAMP-responsive element binding protein. Furthermore, ICER negatively autoregulates the alternative promoter, thus generating a **feedback loop**.
In contrast to the other members of the **CRE**-binding protein family, ICER expression is tissue specific and developmentally regulated. The kinetics of ICER expression are characteristic of an early response gene. Our results indicate that CREM plays a key physiological and developmental role within the hypothalamic-pituitary-gonadal axis. We have previously shown that the transcriptional activator CREM is highly expressed in postmeiotic cells. Spermiogenesis is a complex process by which postmeiotic male germ cells differentiate into mature spermatozoa. This process involves remarkable structural and biochemical changes that are under the hormonal control of the hypothalamic-pituitary axis. We have addressed the specific role of CREM in spermiogenesis using CREM-mutant mice generated by homologous recombination. Analysis of the seminiferous epithelium from mutant male mice reveals that spermatogenesis stops at the first step of spermiogenesis. Late spermatids are completely absent, while there is a significant increase in apoptotic germ cells. A series of postmeiotic germ cell-specific genes are not expressed. Mutant male mice completely lack spermatozoa. This phenotype is reminiscent of cases of

human infertility. We have shown that ICER is regulated in a circadian manner in the pineal gland, the site of the hormone melatonin production. This night-day oscillation is driven by the endogenous clock (located in the suprachiasmatic nucleus, SCN). The synthesis of melatonin is regulated by a rate-limiting enzyme, the serotonin N-acetyltransferase (NAT). By using the CREM-deficient mice and by analysis of the regulatory region of the gene encoding the serotonin NAT, we have established that ICER is responsible for the amplitude and rhythmicity of NAT and thus for the oscillation in the hormonal synthesis of melatonin.

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=> s creb

L4        3561 CREB

=> d his


     (FILE 'HOME' ENTERED AT 14:41:49 ON 02 MAY 2000)

     FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000
L1          128 S CYCLASE AND CRE
L2         2756 S (FEEDBACK LOOP)
L3            1 S L1 AND L2
L4         3561 S CREB

=> s l2 and l4

L5           15 L2 AND L4

=> dup rem l5

PROCESSING COMPLETED FOR L5
L6            9 DUP REM L5 (6 DUPLICATES REMOVED)

=> s reporter

L7        35925 REPORTER

=> s l6 and l7

L8            2 L6 AND L7

=> d ibib abs l8


L8   ANSWER 1 OF 2  MEDLINE
ACCESSION NUMBER:   1999021198     MEDLINE
DOCUMENT NUMBER:    99021198
TITLE:              Inducible cAMP early repressor ICER down-regulation of
                   CREB gene expression in Sertoli cells.
AUTHOR:             Walker W H; Daniel P B; Habener J F
CORPORATE SOURCE:   Laboratory of Molecular Endocrinology, Massachusetts
                    General Hospital, Howard Hughes Medical Institute, Harvard
                    Medical School, Boston 02114, USA.
CONTRACT NUMBER:    DK25532 (NIDDK)
SOURCE:             MOLECULAR AND CELLULAR ENDOCRINOLOGY, (1998 Aug 25) 143
                    (1-2) 167-78.
                    Journal code: E69. ISSN: 0303-7207.
PUB. COUNTRY:       Ireland
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        199903
ENTRY WEEK:         19990301
AB   The cAMP response element binding protein (CREB) and the
     cAMP-responsive element modulator (CREM) are cyclically expressed in the
     seminiferous tubules during spermatogenesis. In the somatic Sertoli
cells,
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which are the major [redacted]porters of germ cell developme[redacted]in the seminiferous tubules, the expression of **CREB** is cycli[redacted] and appears to be regulated by the levels of cAMP produced in response to the pituitary derived follicle-stimulating hormone FSH. Cyclic AMP response elements (CREs) located in the promoter of the **CREB** gene were shown earlier to be implicated in an autopositive **feedback loop** that up-regulates the expression of **CREB**. Here we show that in Sertoli cells FSH-mediated induction of the CREM repressor isoform, ICER (inducible cAMP early repressor) is correlated with the inhibition and delay of **CREB** gene expression in the seminiferous tubules. ICER binds to the two CREs located in the promoter of the **CREB** gene and in transient transfection assays of Sertoli cells, ICER expression vectors down-regulate transcription of a **reporter** gene driven by the **CREB** gene promoter. In addition, analyses of ICER and **CREB** gene expression in isolated segments of rat seminiferous tubules reveals stage-specific and cycle-dependent expression of ICER. The periods of enhanced expression of ICER correspond to the stages of spermatogenesis with the lowest levels of **CREB** expression. We suggest that the expression of ICER in Sertoli cells may contribute to the periodic repression of **CREB** gene expression during the repeated 12-day cycles of spermatogenesis, and may be required to reset the levels of activator **CREB** prior to the initiation of each new cycle of spermatogenesis.

=> d ibib abs 2

L8 ANSWER 2 OF 2 MEDLINE
ACCESSION NUMBER: 93324001 MEDLINE
DOCUMENT NUMBER: 93324001
TITLE: Amplification of calcium-induced gene transcription by nitric oxide in neuronal cells [published erratum appears in Nature 1993 Sep 30;365(6445):468].
AUTHOR: Peunova N; Enikolopov G
CORPORATE SOURCE: Cold Spring Harbor Laboratory, New York 11724.
SOURCE: NATURE, (1993 Jul 29) 364 (6436) 450-3.
Journal code: NSC. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Cancer Journals; Priority Journals
ENTRY MONTH: 199310
AB Nitric oxide (NO) is a short-lived, highly reactive gas, which has been identified as a mediator in vasodilation, an active agent in macrophage cytotoxicity and neurotoxicity, and a neuro-transmitter in the central and peripheral nervous systems. Production of NO by neurons is critical for facilitated synaptic transmission in models of synaptic plasticity such as long-term potentiation and long-term depression, suggesting a role for NO as a retrograde messenger that could complete a hypothetical **feedback loop** by strengthening the connection between postsynaptic and presynaptic cells. We report here that although alone NO has no evident effect on transcription, it can act as an amplifier of calcium signals in neuronal cells. NO and Ca2+ action have to coincide in time for amplification to occur. Experiments with a series of simplified **reporter** genes in combination with specific recombinant protein kinase inhibitors suggest that induction of gene activity following NO-amplified calcium action involves protein kinase A-dependent activation of the transcription factor **CREB**.

=> d his

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FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000

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L1          128 S CYCLASE AND CRE
L2         2756 S (FEEDBACK LOOP)
L3            1 S L1 AND L2
L4         3561 S CREB
L5           15 S L2 AND L4
L6            9 DUP REM L5 (6 DUPLICATES REMOVED)
L7        35925 S REPORTER
L8            2 S L6 AND L7

=> d 15


L5   ANSWER 1 OF 15  MEDLINE
AN   2000053609     MEDLINE
DN   20053609
TI   Activation of Xenopus genes required for lateral inhibition and neuronal
     differentiation during primary neurogenesis.
AU   Koyano-Nakagawa N; Wettstein D; Kintner C
CS   Molecular Neurobiology Laboratory, The Salk Institute for Biological
     Studies, San Diego, California 92186, USA.
SO   MOLECULAR AND CELLULAR NEUROSCIENCES, (1999 Oct-Nov) 14 (4-5) 327-39.
     Journal code: B1D. ISSN: 1044-7431.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200003
EW   20000302


=> d ibib abs 15


L5   ANSWER 1 OF 15  MEDLINE
ACCESSION NUMBER:   2000053609     MEDLINE
DOCUMENT NUMBER:    20053609
TITLE:              Activation of Xenopus genes required for lateral
inhibition
                    and neuronal differentiation during primary neurogenesis.
AUTHOR:             Koyano-Nakagawa N; Wettstein D; Kintner C
CORPORATE SOURCE:   Molecular Neurobiology Laboratory, The Salk Institute for
                    Biological Studies, San Diego, California 92186, USA.
SOURCE:             MOLECULAR AND CELLULAR NEUROSCIENCES, (1999 Oct-Nov) 14
                    (4-5) 327-39.
                    Journal code: B1D. ISSN: 1044-7431.
PUB. COUNTRY:       United States
                    Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200003
ENTRY WEEK:         20000302
AB   XNGN-1, a member of the neurogenin family of basic helix-loop-helix
     proteins, plays a critical role in promoting neuronal differentiation in
     Xenopus embryos. When ectopically expressed, XNGN-1 induces the
expression
     of a set of genes required for neuronal differentiation such as XMyT1 and
     NeuroD. At the same time, however, XNGN-1 induces the expression of genes
     that antagonize neuronal differentiation by a process called lateral
     inhibition. Here, we present evidence that XNGN-1 activates the
expression
     of genes required for differentiation and lateral inhibition by
recruiting
     transcriptional coactivators p300/CBP (CREB-binding protein) or
     PCAF (p300/CBP-associated protein), both of which contain histone
     acetyltransferase (HAT) activity. Significantly, transcriptional
     activation of the genes in the lateral inhibitory pathway is less
     dependent on the HAT activity than is the activation of the genes that
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mediate differentiat[redacted]. We propose that this differe[redacted] enables the genes
in the lateral inhibition pathway to be induced prior to the genes that promote differentiation, thus enabling lateral inhibition to establish a negative **feedback loop** and restrict the number of cells undergoing neuronal differentiation.

=> d ibib abs 1-15 l5

L5 ANSWER 1 OF 15 MEDLINE
ACCESSION NUMBER: 2000053609 MEDLINE
DOCUMENT NUMBER: 20053609
TITLE: Activation of Xenopus genes required for lateral inhibition
and neuronal differentiation during primary neurogenesis.
AUTHOR: Koyano-Nakagawa N; Wettstein D; Kintner C
CORPORATE SOURCE: Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, San Diego, California 92186, USA.
SOURCE: MOLECULAR AND CELLULAR NEUROSCIENCES, (1999 Oct-Nov) 14 (4-5) 327-39.
Journal code: B1D. ISSN: 1044-7431.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY WEEK: 20000302
AB XNGN-1, a member of the neurogenin family of basic helix-loop-helix proteins, plays a critical role in promoting neuronal differentiation in Xenopus embryos. When ectopically expressed, XNGN-1 induces the expression
of a set of genes required for neuronal differentiation such as XMyT1 and NeuroD. At the same time, however, XNGN-1 induces the expression of genes that antagonize neuronal differentiation by a process called lateral inhibition. Here, we present evidence that XNGN-1 activates the expression
of genes required for differentiation and lateral inhibition by recruiting
transcriptional coactivators p300/CBP (**CREB**-binding protein) or PCAF (p300/CBP-associated protein), both of which contain histone acetyltransferase (HAT) activity. Significantly, transcriptional activation of the genes in the lateral inhibitory pathway is less dependent on the HAT activity than is the activation of the genes that mediate differentiation. We propose that this difference enables the genes
in the lateral inhibition pathway to be induced prior to the genes that promote differentiation, thus enabling lateral inhibition to establish a negative **feedback loop** and restrict the number of cells undergoing neuronal differentiation.

L5 ANSWER 2 OF 15 MEDLINE
ACCESSION NUMBER: 1999245828 MEDLINE
DOCUMENT NUMBER: 99245828
TITLE: The Drosophila dCREB2 gene affects the circadian clock.
AUTHOR: Belvin M P; Zhou H; Yin J C
CORPORATE SOURCE: Cold Spring Harbor Laboratory, New York 11724, USA.
CONTRACT NUMBER: 5RO1 NS3557 (NINDS)
1RO1 HL/AR59649 (NHLBI)
SOURCE: NEURON, (1999 Apr) 22 (4) 777-87.
Journal code: AN8. ISSN: 0896-6273.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY WEEK: 19990704
AB We report the role of dCREB2, the Drosophila homolog of **CREB**

/CREM, in circadian [redacted]thms. dCREB2 activity cycles w[redacted] a 24 hr rhythm in flies, both in a light:dark cycle and in constant darkness. A mutation in dCREB2 shortens circadian locomotor rhythm in flies and dampens the oscillation of period, a known clock gene. Cycling dCREB2 activity is abolished in a period mutant, indicating that dCREB2 and Period affect each other and suggesting that the two genes participate in the same regulatory **feedback loop**. We propose that dCREB2 supports cycling of the Period/Timeless oscillator. These findings support **CREB**'s role in mediating adaptive behavioral responses to a variey of environmental stimuli (stress, growth factors, drug addiction, circadian rhythms, and memory formation) in mammals and long-term memory formation and circadian rhythms in Drosophila.

L5 ANSWER 3 OF 15 MEDLINE
ACCESSION NUMBER: 1999071308 MEDLINE
DOCUMENT NUMBER: 99071308
TITLE: **CREB** in the mouse SCN: a molecular interface coding the phase-adjusting stimuli light, glutamate, PACAP, and melatonin for clockwork access.
AUTHOR: von Gall C; Duffield G E; Hastings M H; Kopp M D; Dehghani F; Korf H W; Stehle J H
CORPORATE SOURCE: Dr. Senckenbergische Anatomie, Anatomisches Institut II, Johann Wolfgang Goethe-Universitat, D-60590 Frankfurt, Germany.
SOURCE: JOURNAL OF NEUROSCIENCE, (1998 Dec 15) 18 (24) 10389-97. Journal code: JDF. ISSN: 0270-6474.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY WEEK: 19990404
AB The suprachiasmatic nucleus (SCN) is a central pacemaker in mammals, driving many endogenous circadian rhythms. An important pacemaker target is the regulation of a hormonal message for darkness, the circadian rhythm in melatonin synthesis. The endogenous clock within the SCN is synchronized to environmental light/dark cycles by photic information conveyed via the retinohypothalamic tract (RHT) and by the nocturnal melatonin signal that acts within a **feedback loop**. We investigated how melatonin intersects with the temporally gated resetting actions of two RHT transmitters, pituitary adenylate cyclase-activating polypeptide (PACAP) and glutamate. We analyzed immunocytochemically the inducible phosphorylation of the transcription factor Ca2+/cAMP response element-binding protein (**CREB**) in the SCN of a melatonin-proficient (C3H) and a melatonin-deficient (C57BL) mouse strain. In vivo, light-induced phase shifts in locomotor activity were consistently accompanied by **CREB** phosphorylation in the SCN of both strains. However, in the middle of subjective nighttime, light induced larger phase delays in C57BL than in C3H mice. In vitro, PACAP and glutamate induced **CREB** phosphorylation in the SCN of both mouse strains, with PACAP being more effective during late subjective daytime and glutamate being more effective during subjective nighttime. Melatonin suppressed PACAP- but not glutamate-induced phosphorylation of **CREB**. The distinct temporal domains during which glutamate and PACAP induce **CREB** phosphorylation imply that during the light/dark transition the SCN switches sensitivity between these two RHT transmitters. Because these temporal domains are not different between C3H and C57BL mice, the sensitivity windows are set independently of the rhythmic melatonin signal.

L5 ANSWER 4 OF 15 MEDLINE
ACCESSION NUMBER: 1999021198 MEDLINE

DOCUMENT NUMBER: 99021
TITLE: Inducible cAMP early repressor ICER down-regulation of **CREB** gene expression in Sertoli cells.
AUTHOR: Walker W H; Daniel P B; Habener J F
CORPORATE SOURCE: Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston 02114, USA.
CONTRACT NUMBER: DK25532 (NIDDK)
SOURCE: MOLECULAR AND CELLULAR ENDOCRINOLOGY, (1998 Aug 25) 143 (1-2) 167-78.
Journal code: E69. ISSN: 0303-7207.
PUB. COUNTRY: Ireland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY WEEK: 19990301
AB The cAMP response element binding protein (**CREB**) and the cAMP-responsive element modulator (CREM) are cyclically expressed in the seminiferous tubules during spermatogenesis. In the somatic Sertoli cells, which are the major supporters of germ cell development in the seminiferous tubules, the expression of **CREB** is cyclical and appears to be regulated by the levels of cAMP produced in response to the pituitary derived follicle-stimulating hormone FSH. Cyclic AMP response elements (CREs) located in the promoter of the **CREB** gene were shown earlier to be implicated in an autopositive **feedback loop** that up-regulates the expression of **CREB**. Here we show that in Sertoli cells FSH-mediated induction of the CREM repressor isoform, ICER (inducible cAMP early repressor) is correlated with the inhibition and delay of **CREB** gene expression in the seminiferous tubules. ICER binds to the two CREs located in the promoter of the **CREB** gene and in transient transfection assays of Sertoli cells, ICER expression vectors down-regulate transcription of a reporter gene driven by the **CREB** gene promoter. In addition, analyses of ICER and **CREB** gene expression in isolated segments of rat seminiferous tubules reveals stage-specific and cycle-dependent expression of ICER. The periods of enhanced expression of ICER correspond to the stages of spermatogenesis with the lowest levels of **CREB** expression. We suggest that the expression of ICER in Sertoli cells may contribute to the periodic repression of **CREB** gene expression during the repeated 12-day cycles of spermatogenesis, and may be required to reset the levels of activator **CREB** prior to the initiation of each new cycle of spermatogenesis.

L5 ANSWER 5 OF 15 MEDLINE
ACCESSION NUMBER: 1998335221 MEDLINE
DOCUMENT NUMBER: 98335221
TITLE: The pineal organ, its hormone melatonin, and the photoneuroendocrine system.
AUTHOR: Korf H W; Schomerus C; Stehle J H
CORPORATE SOURCE: Dr. Senckenbergische Anatomie, Anatomisches Institut II, Johann Wolfgang Goethe-Universitat, Frankfurt.
SOURCE: ADVANCES IN ANATOMY, EMBRYOLOGY AND CELL BIOLOGY, (1998) 146 1-100. Ref: 427
Journal code: 2HH. ISSN: 0301-5556.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
ENTRY MONTH: 199810
ENTRY WEEK: 19981002
AB The vertebrate pineal organ rhythmically synthesizes and secretes melatonin during nighttime and forms an essential component of the photoneuroendocrine system which allows humans and animals to measure and keep the time. Regulation of the melatonin biosynthesis depends on signals

from photoreceptors [redacted]ceiving and transmitting envir[redacted]ental light stimuli and endogenous [redacted]oscillators generating a circad[redacted]n rhythm which is independent from any environmental time cue (zeitgeber). In nonmammalian species the photoreceptors responsible for regulating melatonin biosynthesis reside within the pineal organ itself. In several nonmammalian species (e.g., lamprey, zebra fish, house sparrow, chicken) the pineal organ is also capable of generating circadian rhythms and thus serves all key functions of the photoneuroendocrine system: photoreception, endogenous rhythm generation, and production of neurohormones. These may even be accomplished by a single "photoneuroendocrine" cell. In mammals the pineal organ has lost both the direct light sensitivity and the capacity of generating circadian rhythms, and melatonin biosynthesis is regulated by retinal photoreceptors and a circadian oscillator located in the suprachiasmatic nucleus of the hypothalamus. Due to this spatial separation the photoneuroendocrine system of mammals comprises neuronal and neuroendocrine pathways which interconnect its components. The neuronal pathways involve circuits of both the central and the peripheral nervous systems, and as an important final link noradrenergic sympathetic nerve fibers. The suprachiasmatic nucleus appears as a major target of melatonin in mammals. The pineal hormone may thus be involved in a **feedback loop** of the mammalian photoneuroendocrine system. The present comparative contribution considers, after a short survey of classical findings on the phylogenetic development and the gross anatomy of the pineal complex, cytoevolutionary and cell biological aspects of the various types of pinealocytes as well as the afferent and efferent innervation of the pineal organ (pinealofugal and pinealopetal neuronal pathways). Moreover, emphasis is placed on receptor mechanisms, second messenger systems (Ca2+ and cyclic AMP), transcription factors (e.g, **CREB** and ICER), and their roles for regulation of melatonin biosynthesis. Finally, the action, targets, and receptors of melatonin are dealt with. The synoptic approach of this contribution, which combines anatomical and ultrastructural findings with cell and molecular biological results, confirms the functional significance of the melatonin-synthesizing pineal organ as an important component of the photoneuroendocrine system and stresses the importance of this organ as a model to study signal transduction mechanisms both in photoreceptors and in neuroendocrine cells.

L5 ANSWER 6 OF 15 MEDLINE
ACCESSION NUMBER: 1998260039 MEDLINE
DOCUMENT NUMBER: 98260039
TITLE: Coupling gene expression to cAMP signalling: role of **CREB** and CREM.
AUTHOR: Sassone-Corsi P
CORPORATE SOURCE: Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, C.U. de Strasbourg, France.
SOURCE: INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, (1998 Jan) 30 (1) 27-38. Ref: 57
Journal code: CDK. ISSN: 1357-2725.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY WEEK: 19980903
AB Several endocrine and neuronal functions are governed by the cAMP-dependent pathway. Transcriptional regulation upon stimulation of this pathway is mediated by a family of cAMP-responsive nuclear factors. This family consists of a large number of members, which may act as activators or repressors. These factors contain the basic domain/leucine zipper motifs and bind as dimers to cAMP-response elements (CRE). CRE-binding protein (CREBs) function is modulated by phosphorylation by several kinases. Direct activation of gene expression by **CREB**

requires phosphorylation by the cAMP-dependent PKA to serine 133. Among the repressors, ICER (Inducible cAMP Early Repressor) deserves special mention. ICER is generated from an alternative CREM promoter and is the only inducible CRE-binding protein. ICER negatively autoregulates the alternative promoter, generating a **feedback loop**. ICER expression is tissue specific and developmentally regulated. The kinetics of ICER expression are characteristic of an early response gene. CREM plays a key physiological and developmental role within the hypothalamic-pituitary-gonadal axis. The transcriptional activator CREM is highly expressed in postmeiotic cells. The role of CREM in spermiogenesis was addressed using CREM knock-out mice. Spermatogenesis stops at the first step of spermiogenesis in the mutants and there is a significant increase in apoptotic germ cells. This phenotype is reminiscent of cases of human infertility. ICER is regulated in a circadian manner in the pineal gland, the site of the hormone melatonin production. This night-day oscillation is driven by the endogenous clock (located in the suprachiasmatic nucleus). The synthesis of melatonin is regulated by a rate-limiting enzyme, serotonin N-acetyltransferase (NAT). Analysis of the CREM-null mice and of the promoter of the NAT gene revealed that ICER controls the amplitude and rhythmicity of NAT, and thus the oscillation in the hormonal synthesis of melatonin.

L5 ANSWER 7 OF 15 MEDLINE
ACCESSION NUMBER: 97381623 MEDLINE
DOCUMENT NUMBER: 97381623
TITLE: Coupling signalling pathways to transcriptional control: nuclear factors responsive to cAMP.
AUTHOR: Tamai K T; Monaco L; Nantel F; Zazopoulos E; Sassone-Corsi P
CORPORATE SOURCE: Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, Strasbourg, France.
SOURCE: RECENT PROGRESS IN HORMONE RESEARCH, (1997) 52 121-39; discussion 139-40. Ref: 93
Journal code: R1D. ISSN: 0079-9963.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
ENTRY MONTH: 199710

AB Several endocrine and neuronal functions are governed by the cAMP-dependent signalling pathway. In eukaryotes, transcriptional regulation upon stimulation of the adenylyl cyclase signalling pathway is mediated by a family of cAMP-responsive nuclear factors. This family consists of a large number of members that may act as activators or repressors. These factors contain the basic domain/ leucine zipper motifs and bind as dimers to cAMP-response elements (CRE). The function of CRE-binding proteins (CREBs) is modulated by phosphorylation by several kinases. Direct activation of gene expression by **CREB** requires phosphorylation by the cAMP-dependent protein kinase A to the serine-133 residue. Among the repressors, ICER (Inducible cAMP Early Repressor) deserves special mention. ICER is generated from an alternative CREM promoter and constitutes the only inducible cAMP-responsive element binding protein. Furthermore, ICER negatively autoregulates the alternative promoter, thus generating a **feedback loop**.
In contrast to the other members of the CRE-binding protein family, ICER expression is tissue specific and developmentally regulated. The kinetics of ICER expression are characteristic of an early response gene. Our results indicate that CREM plays a key physiological and developmental role within the hypothalamic-pituitary-gonadal axis. We have previously shown that the transcriptional activator CREM is highly expressed in postmeiotic cells. Spermiogenesis is a complex process by which postmeiotic male germ cells differentiate into mature spermatozoa. This process involves remarkable structural and biochemical changes that are under the hormonal control of the hypothalamic-pituitary axis. We have

addressed the specific role of CREM in spermiogenesis using CREM-mutant mice generated by homologous recombination. Analysis of the seminiferous epithelium from mutant male mice reveals that spermatogenesis stops at the first step of spermiogenesis. Late spermatids are completely absent, while there is a significant increase in apoptotic germ cells. A series of postmeiotic germ cell-specific genes are not expressed. Mutant male mice completely lack spermatozoa. This phenotype is reminiscent of cases of human infertility. We have shown that ICER is regulated in a circadian manner in the pineal gland, the site of the hormone melatonin production. This night-day oscillation is driven by the endogenous clock (located in the suprachiasmatic nucleus, SCN). The synthesis of melatonin is regulated by a rate-limiting enzyme, the serotonin N-acetyltransferase (NAT). By using the CREM-deficient mice and by analysis of the regulatory region of the gene encoding the serotonin NAT, we have established that ICER is responsible for the amplitude and rhythmicity of NAT and thus for the oscillation in the hormonal synthesis of melatonin.

L5 ANSWER 8 OF 15 MEDLINE
ACCESSION NUMBER: 93324001 MEDLINE
DOCUMENT NUMBER: 93324001
TITLE: Amplification of calcium-induced gene transcription by nitric oxide in neuronal cells [published erratum appears in Nature 1993 Sep 30;365(6445):468].
AUTHOR: Peunova N; Enikolopov G
CORPORATE SOURCE: Cold Spring Harbor Laboratory, New York 11724.
SOURCE: NATURE, (1993 Jul 29) 364 (6436) 450-3.
Journal code: NSC. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Cancer Journals; Priority Journals
ENTRY MONTH: 199310
AB Nitric oxide (NO) is a short-lived, highly reactive gas, which has been identified as a mediator in vasodilation, an active agent in macrophage cytotoxicity and neurotoxicity, and a neuro-transmitter in the central and peripheral nervous systems. Production of NO by neurons is critical for facilitated synaptic transmission in models of synaptic plasticity such as long-term potentiation and long-term depression, suggesting a role for NO as a retrograde messenger that could complete a hypothetical **feedback loop** by strengthening the connection between postsynaptic and presynaptic cells. We report here that although alone NO has no evident effect on transcription, it can act as an amplifier of calcium signals in neuronal cells. NO and Ca2+ action have to coincide in time for amplification to occur. Experiments with a series of simplified reporter genes in combination with specific recombinant protein kinase inhibitors suggest that induction of gene activity following NO-amplified calcium action involves protein kinase A-dependent activation of the transcription factor **CREB**.

L5 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 2000:9222 BIOSIS
DOCUMENT NUMBER: PREV200000009222
TITLE: Activation of Xenopus genes required for lateral inhibition and neuronal differentiation during primary neurogenesis.
AUTHOR(S): Koyano-Nakagawa, Naoko; Wettstein, Daniel; Kintner, Chris (1)
CORPORATE SOURCE: (1) Molecular Neurobiology Laboratory, Salk Institute for Biological Studies, San Diego, CA, 92186 USA
SOURCE: Molecular and Cellular Neuroscience, (Oct. Nov., 1999) Vol. 14, No. 4-5, pp. 327-339.
ISSN: 1044-7431.
DOCUMENT TYPE: Article

LANGUAGE: Englis
SUMMARY LANGUAGE: English
AB XNGN-1, a member of the neurogenin family of basic helix-loop-helix proteins, plays a critical role in promoting neuronal differentiation in Xenopus embryos. When ectopically expressed, XNGN-1 induces the expression of a set of genes required for neuronal differentiation such as XMyT1 and NeuroD. At the same time, however, XNGN-1 induces the expression of genes that antagonize neuronal differentiation by a process called lateral inhibition. Here, we present evidence that XNGN-1 activates the expression of genes required for differentiation and lateral inhibition by recruiting transcriptional coactivators p300/CBP (**CREB**-binding protein) or PCAF (p300/CBP-associated protein), both of which contain histone acetyltransferase (HAT) activity. Significantly, transcriptional activation of the genes in the lateral inhibitory pathway is less dependent on the HAT activity than is the activation of the genes that mediate differentiation. We propose that this difference enables the genes in the la teral inhibition pathway to be induced prior to the genes that promote differentiation, thus enabling lateral inhibition to establish a negative **feedback loop** and restrict the number of cells undergoing neuronal differentiation.

L5 ANSWER 10 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:248178 BIOSIS
DOCUMENT NUMBER: PREV199900248178
TITLE: The Drosophila dCREB2 gene affects the circadian clock.
AUTHOR(S): Belvin, Marcia P.; Zhou, Hong; Yin, Jerry C. P. (1)
CORPORATE SOURCE: (1) Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY, 11724 USA
SOURCE: Neuron, (April, 1999) Vol. 22, No. 4, pp. 777-787.
ISSN: 0896-6273.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We report the role of dCREB2, the Drosophila homolog of **CREB**/CREM, in circadian rhythms. dCREB2 activity cycles with a 24 hr rhythm in flies, both in a light:dark cycle and in constant darkness. A mutation in dCREB2 shortens circadian locomotor rhythm in flies and dampens the oscillation of period, a known clock gene. Cycling dCREB2 activity is abolished in a period mutant, indicating that dCREB2 and Period affect each other and suggesting that the two genes participate in the same regulatory **feedback loop**. We propose that dCREB2 supports cycling of the Period/Timeless oscillator. These findings support **CREB**'s role in mediating adaptive behavioral responses to a variey of environmental stimuli (stress, growth factors, drug addiction, circadian rhythms, and memory formation) in mammals and long-term memory formation and circadian rhythms in Drosophila.

L5 ANSWER 11 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:111637 BIOSIS
DOCUMENT NUMBER: PREV199900111637
TITLE: Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1.
AUTHOR(S): Bhattacharya, Shoumo; Michels, Catherine L.; Leung, Man-Kit; Arany, Zoltan P.; Kung, Andrew L.; Livingston, David M. (1)
CORPORATE SOURCE: (1) Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115 USA
SOURCE: Genes & Development, (Jan. 1, 1999) Vol. 13, No. 1, pp. 64-75.
ISSN: 0890-9369.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Recruitment of p300/CBP by the hypoxia-inducible factor, HIF-1, is

essential for the transcriptional response to hypoxia and requires an interaction between the p300/CBP CH1 region and HIF-1alpha. A new p300-CH1
interacting protein, p35srj, has been identified and cloned. p35srj is an alternatively spliced isoform of MRG1, a human protein of unknown function. Virtually all endogenous p35srj is bound to p300/CBP in vivo, and it inhibits HIF-1 transactivation by blocking the HIF-1alpha/p300 CH1 interaction. p35srj did not affect transactivation by transcription factors that bind p300/CBP outside the CHI region. Endogenous p35srj is up-regulated markedly by the HIF-1 activators hypoxia or deferoxamine, suggesting that it could operate in a negative-**feedback loop**. In keeping with this notion, a p300 CH1 mutant domain, defective in HIF-1 but not p35srj binding, enhanced endogenous HIF-1 function. In hypoxic cells, p35srj may regulate HIF-1 transactivation by controlling access of HIF-1alpha to p300/CBP, and may keep a significant portion of p300/CBP available for interaction with other transcription factors by partially sequestering and functionally compartmentalizing cellular p300/CBP.

L5 ANSWER 12 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1999:53065 BIOSIS
DOCUMENT NUMBER: PREV199900053065
TITLE: **CREB** in the mouse SCN: A molecular interface coding the phase-adjusting stimuli light, glutamate, PACAP,
and melatonin for clockwork access.
AUTHOR(S): von Gall, Charlotte; Duffield, Giles E.; Hastings, Michael H.; Kopp, Michael D. A.; Dehghani, Faramarz; Korf, Horst-Werner; Stehle, Joerg H. (1)
CORPORATE SOURCE: (1) Dr. Senckenbergische Anat., Anat. Inst. II, Johann Wolfgang Goethe-Univ., Theodor-Stern-Kai 7, D-60590 Frankfurt Germany
SOURCE: Journal of Neuroscience, (Dec. 15, 1998) Vol. 18, No. 24, pp. 10389-10397.
ISSN: 0270-6474.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The suprachiasmatic nucleus (SCN) is a central pacemaker in mammals, driving many endogenous circadian rhythms. An important pacemaker target is the regulation of a hormonal message for darkness, the circadian rhythm
in melatonin synthesis. The endogenous clock within the SCN is synchronized to environmental light/dark cycles by photic information conveyed via the retinohypothalamic tract (RHT) and by the nocturnal melatonin signal that acts within a **feedback loop**. We investigated how melatonin intersects with the temporally gated resetting actions of two RHT transmitters, pituitary adenylate cyclase-activating polypeptide (PACAP) and glutamate. We analyzed immunocytochemically the inducible phosphorylation of the transcription factor Ca2+/cAMP response element-binding protein (**CREB**) in the SCN of a melatonin-proficient (C3H) and a melatonin-deficient (C57BL) mouse strain.
In vivo, light-induced phase shifts in locomotor activity were consistently accompanied by **CREB** phosphorylation in the SCN of both strains. However, in the middle of subjective nighttime, light induced larger phase delays in C57BL than in C3H mice. In vitro, PACAP and
glutamate induced **CREB** phosphorylation in the SCN of both mouse strains, with PACAP being more effective during late subjective daytime and glutamate being more effective during subjective nighttime. Melatonin suppressed PACAP- but not glutamate-induced phosphorylation of **CREB**. The distinct temporal domains during which glutamate and PACAP induce **CREB** phosphorylation imply that during the light/dark transition the SCN switches sensitivity between these two RHT transmitters. Because these temporal domains are not different between C3H
and C57BL mice, the sensitivity windows are set independently of the rhythmic melatonin signal.

L5 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1998:503036 BIOSIS
DOCUMENT NUMBER: PREV199800503036
TITLE: Inducible cAMP early repressor ICER down-regulation of **CREB** gene expression in Sertoli cells.
AUTHOR(S): Walker, William H.; Daniel, Philip B.; Habener, Joel F. (1)
CORPORATE SOURCE: (1) Lab. Mol. Endocrinol., Mass. Gen. Hosp., Howard Hughes Med. Inst., Harvard Med. Sch., Wellman 320, Boston, MA 02114 USA
SOURCE: Molecular and Cellular Endocrinology, (Aug. 25, 1998) Vol. 143, No. 1-2, pp. 167-178.
ISSN: 0303-7207.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The cAMP response element binding protein (**CREB**) and the cAMP-responsive element modulator (CREM) are cyclically expressed in the seminiferous tubules during spermatogenesis. In the somatic Sertoli cells, which are the major supporters of germ cell development in the seminiferous tubules, the expression of **CREB** is cyclical and appears to be regulated by the levels of cAMP produced in response to the pituitary derived follicle-stimulating hormone FSH. Cyclic AMP response elements (CREs) located in the promoter of the **CREB** gene were shown earlier to be implicated in an autopositive **feedback loop** that up-regulates the expression of **CREB**. Here we show that in Sertoli cells FSH-mediated induction of the CREM repressor isoform, ICER (inducible cAMP early repressor) is correlated with the inhibition and delay of **CREB** gene expression in the seminiferous tubules. ICER binds to the two CREs located in the promoter of the **CREB** gene and in transient transfection assays of Sertoli cells, ICER expression vectors down-regulate transcription of a reporter gene driven by the **CREB** gene promoter. In addition, analyses of ICER and **CREB** gene expression in isolated segments of rat seminiferous tubules reveals stage-specific and cycle-dependent expression of ICER. The periods of enhanced expression of ICER correspond to the stages of spermatogenesis with the lowest levels of **CREB** expression. We suggest that the expression of ICER in Sertoli cells may contribute to the periodic repression of **CREB** gene expression during the repeated 12-day cycles of spermatogenesis, and may be required to reset the levels of activator **CREB** prior to the initiation of each new cycle of spermatogenesis.

L5 ANSWER 14 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1998:256848 BIOSIS
DOCUMENT NUMBER: PREV199800256848
TITLE: Coupling gene expression to cAMP signalling: Role of **CREB** and CREM.
AUTHOR(S): Sassone-Corsi, Paolo (1)
CORPORATE SOURCE: (1) Inst. Genet. Biol. Mol. Cell., BP 163, CU de Strasbourg, 67404 Illkirch-Cedex France
SOURCE: International Journal of Biochemistry & Cell Biology, (Jan., 1998) Vol. 30, No. 1, pp. 27-38.
ISSN: 1357-2725.
DOCUMENT TYPE: General Review
LANGUAGE: English
AB Several endocrine and neuronal functions are governed by the CAMP-dependent pathway. Transcriptional regulation upon stimulation of this pathway is mediated by a family of cAMP-responsive nuclear factors. This family consists of a large number of members, which may act as activators or repressors. These factors contain the basic domain/leucine zipper motifs and bind as dimers to cAMP-response elements (CRE). CRE-binding protein (CREBs) function is modulated by phosphorylation by several kinases. Direct activation of gene expression by **CREB** requires phosphorylation by the cAMP-dependent PKA to serine 133. Among the repressors, ICER (Inducible CAMP Early Repressor) deserves special mention. ICER is generated from an alternative CREM promoter and is the only inducible CRE-binding protein. ICER negatively autoregulates the

alternative promoter, generating a **feedback loop**. ICER expression is tissue specific and developmentally regulated. The kinetics of ICER expression are characteristic of an early response gene. CREM plays a key physiological and developmental role within the hypothalamic-pituitary-gonadal axis. The transcriptional activator CREM is highly expressed in postmeiotic cells. The role of CREM in spermiogenesis was addressed using CREM knock-out mice. Spermatogenesis stops at the first step of spermiogenesis in the mutants and there is a significant increase in apoptotic germ cells. This phenotype is reminiscent of cases of human infertility. ICER is regulated in a circadian manner in the pineal gland, the site of the hormone melatonin production. This night-day oscillation is driven by the endogenous clock (located in the suprachiasmatic nucleus). The synthesis of melatonin is regulated by a rate-limiting enzyme, serotonin N-acetyltransferase (NAT). Analysis of the CREM-null mice and of the promoter of the NAT gene revealed that ICER controls the amplitude and rhythmicity of NAT, and thus the oscillation in the hormonal synthesis of melatonin.

L5 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1993:432559 BIOSIS
DOCUMENT NUMBER: PREV199396087184
TITLE: Amplification of calcium-induced gene transcription by nitric oxide in neuronal cells.
AUTHOR(S): Peunova, Natalia; Enikolopov, Grigori (1)
CORPORATE SOURCE: (1) Cold Spring Harbor Lab., P.O. Box 100, Cold Spring Harbor, NY 11724 USA
SOURCE: Nature (London), (1993) Vol. 364, No. 6436, pp. 450-453. ISSN: 0028-0836.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Nitric oxide (NO) is a short-lived, highly reactive gas, which has been identified as a mediator in vasodilation, an active agent in macrophage cytotoxicity and neurotoxicity, and a neurotransmitter in the central and peripheral nervous systems-1-5. Production of NO by neurons is critical for facilitated synaptic transmission in models of synaptic plasticity such as long-term potentiation and long-term depression, suggesting a role for NO as a retrograde messenger that could complete a hypothetical **feedback loop** by strengthening the connection between postsynaptic and presynaptic cells-6-10. We report here that although alone NO has no evident effect on transcription, it can act as an amplifier of calcium signals in neuronal cells. NO and Ca-2+ action have to coincide in time for amplification to occur. Experiments with a series of simplified reporter genes in combination with specific recombinant protein kinase inhibitors suggest that induction of gene activity following NO-amplified calcium action involves protein kinase A-dependent activation of the transcription factor **CREB**.

=> s autopositive

L9 4 AUTOPOSITIVE

=> s auto-feedback

L10 30 AUTO-FEEDBACK

=> s amplification

L11 77963 AMPLIFICATION

=> s amplif?

L12 131206 AMPLIF?

```
=> s reporter assay

L13         276 REPORTER ASSAY

=> d his


     (FILE 'HOME' ENTERED AT 14:41:49 ON 02 MAY 2000)

     FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000
L1           128 S CYCLASE AND CRE
L2          2756 S (FEEDBACK LOOP)
L3             1 S L1 AND L2
L4          3561 S CREB
L5            15 S L2 AND L4
L6             9 DUP REM L5 (6 DUPLICATES REMOVED)
L7         35925 S REPORTER
L8             2 S L6 AND L7
L9             4 S AUTOPOSITIVE
L10           30 S AUTO-FEEDBACK
L11        77963 S AMPLIFICATION
L12       131206 S AMPLIF?
L13          276 S REPORTER ASSAY

=> l2 or l9 or l10

L2 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l2 or l9 or l10

L14        2788 L2 OR L9 OR L10

=> s l13 and l14

L15           0 L13 AND L14

=> s l14 and reporter

L16          47 L14 AND REPORTER

=> dup rem l16

PROCESSING COMPLETED FOR L16
L17           28 DUP REM L16 (19 DUPLICATES REMOVED)

=> s l14 and l17

L18          28 L14 AND L17

=> s l17 and l12

L19           3 L17 AND L12

=> d ibib abs 1-3


L19  ANSWER 1 OF 3  MEDLINE
ACCESSION NUMBER:   97294636     MEDLINE
DOCUMENT NUMBER:    97294636
TITLE:              Tolerance of high levels of wild-type p53 in transformed
                    epithelial cells dependent on auto-regulation by mdm-2.
AUTHOR:             Blaydes J P; Gire V; Rowson J M; Wynford-Thomas D
CORPORATE SOURCE:   Department of Pathology, University of Wales College of
                    Medicine, Cardiff, UK.
SOURCE:             ONCOGENE, (1997 Apr 17) 14 (15) 1859-68.
                    Journal code: ONC. ISSN: 0950-9232.
```

PUB. COUNTRY: ENGLAND United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199708
ENTRY WEEK: 19970802

AB A significant proportion of human cancers express high levels of p53 protein in the absence of an underlying mutation in the gene. Using transformed (Vh1) and non-transformed (FRTL-5) rat thyroid epithelial cell lines as a model, we have examined the mechanisms by which high levels of wild-type p53 may be tolerated. Stable transfection with p53-dependent **reporter** constructs demonstrated that the 'excess' wild-type p53 in Vh1 cells is not associated with a comparable increase in p53-dependent transcription (though the response to u.v. irradiation is retained). Mdm-2, which binds p53 and inhibits its transactivation activity, is overexpressed in Vh1 cells in the absence of gene **amplification** and in a p53-dependent manner. Furthermore disruption of p53-mdm-2 complex formation in Vh1 cells by microinjection of an antibody to the p53-binding domain of mdm-2 resulted in a dramatic increase in p53-dependent transcription. Since only a small proportion of the p53 in Vh1 cells was found to be in complex with mdm-2 (the majority of unbound protein being in a latent form), this suggests that mdm-2 selectively binds a pool of p53 that would otherwise be active as a sequence-specific activator of transcription. We suggest that, in some types of tumour, the 'sensitivity' of the p53-driven mdm-2 **feedback loop** may be sufficient to prevent free, active p53 reaching the level required for growth arrest or apoptosis, making them an ideal target for therapies designed to disrupt p53-mdm-2 interactions.

L19 ANSWER 2 OF 3 MEDLINE
ACCESSION NUMBER: 94195807 MEDLINE
DOCUMENT NUMBER: 94195807
TITLE: Interactions between p53 and MDM2 in a mammalian cell cycle checkpoint pathway.
AUTHOR: Chen C Y; Oliner J D; Zhan Q; Fornace A J Jr; Vogelstein B; Kastan M B
CORPORATE SOURCE: Johns Hopkins Oncology Center, Baltimore, MD 21287.
CONTRACT NUMBER: ES05777 (NIEHS)
CA43460 (NCI)
CA41183 (NCI)
+
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Mar 29) 91 (7) 2684-8.
Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199407

AB Normal p53 function is required for optimal arrest of cells in the G1 phase of the cell cycle following certain types of DNA damage. Loss of this cell cycle checkpoint may contribute to tumor development by increasing the number of genetic abnormalities in daughter cells following DNA damage. The MDM2 protein is an endogenous gene product that binds to the p53 protein and is able to block p53-mediated transactivation of cotransfected **reporter** constructs; thus, interactions between MDM2 and p53 in this checkpoint pathway following ionizing irradiation were examined. Though increases in p53 protein by DNA damage were not abrogated by MDM2 overexpression, increased levels of MDM2, resulting either from endogenous gene **amplification** or from transfection of an exogenous expression vector, were associated with a reduction in the

ability of cells to arrest in G1 following irradiation. In addition, expression of endogenous MDM2 was enhanced by ionizing irradiation at the level of transcription in a p53-dependent fashion. These observations demonstrate that MDM2 overexpression can inhibit p53 function in a known physiologic pathway and are consistent with the hypothesis that MDM2 may function in a "**feedback loop**" mechanism with p53, possibly acting to limit the length or severity of the p53-mediated arrest following DNA damage.

L19 ANSWER 3 OF 3 MEDLINE
ACCESSION NUMBER: 93324001 MEDLINE
DOCUMENT NUMBER: 93324001
TITLE: **Amplification** of calcium-induced gene transcription by nitric oxide in neuronal cells [published erratum appears in Nature 1993 Sep 30;365(6445):468].
AUTHOR: Peunova N; Enikolopov G
CORPORATE SOURCE: Cold Spring Harbor Laboratory, New York 11724.
SOURCE: NATURE, (1993 Jul 29) 364 (6436) 450-3.
Journal code: NSC. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Cancer Journals; Priority Journals
ENTRY MONTH: 199310
AB Nitric oxide (NO) is a short-lived, highly reactive gas, which has been identified as a mediator in vasodilation, an active agent in macrophage cytotoxicity and neurotoxicity, and a neuro-transmitter in the central and peripheral nervous systems. Production of NO by neurons is critical for facilitated synaptic transmission in models of synaptic plasticity such as long-term potentiation and long-term depression, suggesting a role for NO as a retrograde messenger that could complete a hypothetical **feedback loop** by strengthening the connection between postsynaptic and presynaptic cells. We report here that although alone NO has no evident effect on transcription, it can act as an **amplifier** of calcium signals in neuronal cells. NO and Ca2+ action have to coincide in time for **amplification** to occur. Experiments with a series of simplified **reporter** genes in combination with specific recombinant protein kinase inhibitors suggest that induction of gene activity following NO-**amplified** calcium action involves protein kinase A-dependent activation of the transcription factor CREB.

=> d his

(FILE 'HOME' ENTERED AT 14:41:49 ON 02 MAY 2000)

FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000

L1	128	S CYCLASE AND CRE
L2	2756	S (FEEDBACK LOOP)
L3	1	S L1 AND L2
L4	3561	S CREB
L5	15	S L2 AND L4
L6	9	DUP REM L5 (6 DUPLICATES REMOVED)
L7	35925	S REPORTER
L8	2	S L6 AND L7
L9	4	S AUTOPOSITIVE
L10	30	S AUTO-FEEDBACK
L11	77963	S AMPLIFICATION
L12	131206	S AMPLIF?
L13	276	S REPORTER ASSAY
L14	2788	S L2 OR L9 OR L10
L15	0	S L13 AND L14
L16	47	S L14 AND REPORTER
L17	28	DUP REM L16 (19 DUPLICATES REMOVED)
L18	28	S L14 AND L17

L19 3 S L17 AND [redacted]

=> s reporter construct?

L20 3124 REPORTER CONSTRUCT?

=> s l11

L21 77963 L11

=> s l11 and l20

L22 77 L11 AND L20

=> s l22 and l14

L23 4 L22 AND L14

=> dup rem l23

PROCESSING COMPLETED FOR L23
L24 2 DUP REM L23 (2 DUPLICATES REMOVED)

=> d ibib abs

L24 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 97294636 MEDLINE
DOCUMENT NUMBER: 97294636
TITLE: Tolerance of high levels of wild-type p53 in transformed epithelial cells dependent on auto-regulation by mdm-2.
AUTHOR: Blaydes J P; Gire V; Rowson J M; Wynford-Thomas D
CORPORATE SOURCE: Department of Pathology, University of Wales College of Medicine, Cardiff, UK.
SOURCE: ONCOGENE, (1997 Apr 17) 14 (15) 1859-68.
Journal code: ONC. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199708
ENTRY WEEK: 19970802

AB A significant proportion of human cancers express high levels of p53 protein in the absence of an underlying mutation in the gene. Using transformed (Vh1) and non-transformed (FRTL-5) rat thyroid epithelial cell lines as a model, we have examined the mechanisms by which high levels of wild-type p53 may be tolerated. Stable transfection with p53-dependent **reporter constructs** demonstrated that the 'excess' wild-type p53 in Vh1 cells is not associated with a comparable increase in p53-dependent transcription (though the response to u.v. irradiation is retained). Mdm-2, which binds p53 and inhibits its transactivation activity, is overexpressed in Vh1 cells in the absence of gene **amplification** and in a p53-dependent manner. Furthermore disruption of p53-mdm-2 complex formation in Vh1 cells by microinjection of an antibody to the p53-binding domain of mdm-2 resulted in a dramatic increase in p53-dependent transcription. Since only a small proportion of the p53 in Vh1 cells was found to be in complex with mdm-2 (the majority of unbound protein being in a latent form), this suggests that mdm-2 selectively binds a pool of p53 that would otherwise be active as a sequence-specific activator of transcription. We suggest that, in some types of tumour, the 'sensitivity' of the p53-driven mdm-2 **feedback loop** may be sufficient to prevent free, active p53 reaching the level required for growth arrest or apoptosis, making them an ideal target for therapies designed to disrupt p53-mdm-2 interactions.

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=> d his

     (FILE 'HOME' ENTERED AT 14:41:49 ON 02 MAY 2000)

     FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000
L1           128 S CYCLASE AND CRE
L2          2756 S (FEEDBACK LOOP)
L3             1 S L1 AND L2
L4          3561 S CREB
L5            15 S L2 AND L4
L6             9 DUP REM L5 (6 DUPLICATES REMOVED)
L7         35925 S REPORTER
L8             2 S L6 AND L7
L9             4 S AUTOPOSITIVE
L10           30 S AUTO-FEEDBACK
L11        77963 S AMPLIFICATION
L12       131206 S AMPLIF?
L13          276 S REPORTER ASSAY
L14         2788 S L2 OR L9 OR L10
L15            0 S L13 AND L14
L16           47 S L14 AND REPORTER
L17           28 DUP REM L16 (19 DUPLICATES REMOVED)
L18           28 S L14 AND L17
L19            3 S L17 AND L12
L20         3124 S REPORTER CONSTRUCT?
L21        77963 S L11
L22           77 S L11 AND L20
L23            4 S L22 AND L14
L24            2 DUP REM L23 (2 DUPLICATES REMOVED)

=> d ibib absl l17

'ABSL' IS NOT A VALID FORMAT
In a multifile environment, a format can only be used if it is valid
in at least one of the files.  Refer to file specific help messages
or the STNGUIDE file for information on formats available in
individual files.
REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):

REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):end


=> d ibib abs l17


L17  ANSWER 1 OF 28  MEDLINE
ACCESSION NUMBER:    2000129922      MEDLINE
DOCUMENT NUMBER:     20129922
TITLE:               Normal limb development in conditional mutants of Fgf4.
AUTHOR:              Moon A M; Boulet A M; Capecchi M R
CORPORATE SOURCE:    Howard Hughes Medical Institute, Department of Human
                     Genetics, University of Utah School of Medicine, Salt Lake
                     City, UT 84112, USA.
SOURCE:              DEVELOPMENT, (2000 Mar) 127 (5) 989-96.
                     Journal code: ECW. ISSN: 0950-1991.
PUB. COUNTRY:        ENGLAND: United Kingdom
                     Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         200006
ENTRY WEEK:          20000602
AB   Fibroblast growth factors (FGFs) mediate multiple developmental signals
in
     vertebrates. Several of these factors are expressed in limb bud
structures
     that direct patterning of the limb. FGF4 is produced in the apical
     ectodermal ridge (AER) where it is hypothesized to provide mitogenic and
     morphogenic signals to the underlying mesenchyme that regulate normal
limb
```

development. Mutation of this gene in the germline of mice results in early embryonic lethality, preventing subsequent evaluation of Fgf4 function in the AER. A conditional mutant of Fgf4, based on site-specific Cre/loxP-mediated excision of the gene, allowed us to bypass embryonic lethality and directly test the role of FGF4 during limb development in living murine embryos. This conditional mutation was designed so that concomitant with inactivation of the Fgf4 gene by excision of all Fgf4-coding sequences, a **reporter** gene was activated in Fgf4-expressing cells, allowing assessment of the site-specific recombination reaction. Although a large body of evidence led us to predict that ablation of Fgf4 gene function in the AER of developing mice would result in abnormal limb outgrowth and patterning, we found that Fgf4 conditional mutants had normal limbs. Furthermore, expression patterns of Shh, Bmp2, Fgf8 and Fgf10 were normal in the limb buds of the conditional mutants. These findings indicate that the previously proposed FGF4-SHH **feedback loop** is not essential for coordination of murine limb outgrowth and patterning. We suggest that some of the roles currently attributed to FGF4 during early vertebrate limb development may be performed by other AER factors in vivo.

=> d his

(FILE 'HOME' ENTERED AT 14:41:49 ON 02 MAY 2000)

FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000

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L1          128 S CYCLASE AND CRE
L2         2756 S (FEEDBACK LOOP)
L3            1 S L1 AND L2
L4         3561 S CREB
L5           15 S L2 AND L4
L6            9 DUP REM L5 (6 DUPLICATES REMOVED)
L7        35925 S REPORTER
L8            2 S L6 AND L7
L9            4 S AUTOPOSITIVE
L10          30 S AUTO-FEEDBACK
L11       77963 S AMPLIFICATION
L12      131206 S AMPLIF?
L13         276 S REPORTER ASSAY
L14        2788 S L2 OR L9 OR L10
L15           0 S L13 AND L14
L16          47 S L14 AND REPORTER
L17          28 DUP REM L16 (19 DUPLICATES REMOVED)
L18          28 S L14 AND L17
L19           3 S L17 AND L12
L20        3124 S REPORTER CONSTRUCT?
L21       77963 S L11
L22          77 S L11 AND L20
L23           4 S L22 AND L14
L24           2 DUP REM L23 (2 DUPLICATES REMOVED)
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=> s l1 and l12

L25 1 L1 AND L12

=> d ibib abs

L25 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2000 BIOSIS

ACCESSION NUMBER: 2000:108760 BIOSIS

DOCUMENT NUMBER: PREV200000108760

TITLE: Essentiality of intron control in the induction of c-fos by glucose and glucoincretin peptides in INS-1 beta-cells.

AUTHOR(S): Susini, Stefan; Van Haasteren, Goedele (1); Li, Senlin; Prentki, Marc; Schlegel, Werner

CORPORATE SOURCE: (1) Fondation pour Recherches Medicales, University of

Gene[redacted] 64 Avenue de la Roseraie, 1211 Geneva Switzerland
SOURCE: FASEB Journal, (Jan., 2000) Vol. 14, No. 1, pp. 128-136.
ISSN: 0892-6638.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Glucose controls long-term processes in the pancreatic beta-cell such as metabolic enzymes gene expression, cell growth, and apoptosis. Such control is likely mediated via the expression of immediate-early response genes since several of these genes including c-fos are strongly induced by glucose in the beta-cell line INS-1, provided costimulation with cAMP-raising glucoincretin hormones. This study addresses the mechanism of c-fos gene activation by glucose. Glucose in the presence of chlorophenylthio-cAMP generated a low threefold induction of the c-fos/basic luciferase reporter gene, which includes only the c-fos promoter. In contrast, the c-fos/intron construct containing the first intron in addition to promoter elements showed a pronounced 16-fold induction, comparable to the increased c-fos mRNA accumulation. Similar observations were made with glucose in combination with the glucoincretins glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide, and pituitary adenylyl **cyclase**-activating peptide 38. Deletion of a 119 bp region in intron 1 that includes a transcriptional arrest site did not affect the inductive process. In contrast, a 534 bp deletion comprising a major part of the intron reduced the induction by 75%. At the promoter level, mutating the cAMP response element reduced by more than 60% the transcriptional activation whereas mutating the serum response element had no effect. Inhibitors of protein kinase A and Ca2+/calmodulin-dependent protein kinases each reduced by 50% the reporter gene activation and together fully prevented the glucose-glucoincretin effect. In conclusion, the strong induction of c-fos by glucose and glucoincretins results from Ca2+ and cAMP signaling pathways addressing both the **CRE** in the promoter and essential response element(s) in the first intron that are unrelated to the transcription arrest site.

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(FILE 'HOME' ENTERED AT 14:41:49 ON 02 MAY 2000)

FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000

L1	128	S	CYCLASE AND CRE
L2	2756	S	(FEEDBACK LOOP)
L3	1	S	L1 AND L2
L4	3561	S	CREB
L5	15	S	L2 AND L4
L6	9	DUP REM	L5 (6 DUPLICATES REMOVED)
L7	35925	S	REPORTER
L8	2	S	L6 AND L7
L9	4	S	AUTOPOSITIVE
L10	30	S	AUTO-FEEDBACK
L11	77963	S	AMPLIFICATION
L12	131206	S	AMPLIF?
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L14	2788	S	L2 OR L9 OR L10
L15	0	S	L13 AND L14
L16	47	S	L14 AND REPORTER
L17	28	DUP REM	L16 (19 DUPLICATES REMOVED)
L18	28	S	L14 AND L17
L19	3	S	L17 AND L12
L20	3124	S	REPORTER CONSTRUCT?
L21	77963	S	L11
L22	77	S	L11 AND L20

L23 4 S L22 AND L14
L24 2 DUP REM L23 (2 DUPLICATES REMOVED)
L25 1 S L1 AND L12

=> s l1 and l7

L26 37 L1 AND L7

=> s l26 and l16

L27 0 L26 AND L16

=> s l26 and l14

L28 0 L26 AND L14

=> dup rem l26

PROCESSING COMPLETED FOR L26
L29 20 DUP REM L26 (17 DUPLICATES REMOVED)

=> d ibib abs 1-20

L29 ANSWER 1 OF 20 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000123912 MEDLINE
DOCUMENT NUMBER: 20123912
TITLE: Essentiality of intron control in the induction of c-fos by glucose and glucoincretin peptides in INS-1 beta-cells.
AUTHOR: Susini S; Van Haasteren G; Li S; Prentki M; Schlegel W
CORPORATE SOURCE: Fondation pour Recherches Medicales, University of Geneva, 1211 Geneva, Switzerland.
SOURCE: FASEB JOURNAL, (2000 Jan) 14 (1) 128-36.
Journal code: FAS. ISSN: 0892-6638.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 200004
ENTRY WEEK: 20000403
AB Glucose controls long-term processes in the pancreatic beta-cell such as metabolic enzymes gene expression, cell growth, and apoptosis. Such control is likely mediated via the expression of immediate-early response genes since several of these genes including c-fos are strongly induced by glucose in the beta-cell line INS-1, provided costimulation with cAMP-raising glucoincretin hormones. This study addresses the mechanism of c-fos gene activation by glucose. Glucose in the presence of chlorophenylthio-cAMP generated a low threefold induction of the c-fos/basic luciferase **reporter** gene, which includes only the c-fos promoter. In contrast, the c-fos/intron construct containing the first intron in addition to promoter elements showed a pronounced 16-fold induction, comparable to the increased c-fos mRNA accumulation. Similar observations were made with glucose in combination with the glucoincretins glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide, and pituitary adenylyl **cyclase**-activating peptide 38. Deletion of a 119 bp region in intron 1 that includes a transcriptional arrest site did not affect the inductive process. In contrast, a 534 bp deletion comprising a major part of the intron reduced the induction by 75%. At the promoter level, mutating the cAMP response element reduced by more than 60% the transcriptional activation whereas mutating the serum response element had no effect. Inhibitors of protein kinase A and Ca(2+)/calmodulin-dependent protein kinases each reduced by 50% the **reporter** gene activation and together fully prevented the

glucose-glucoincret[redacted]effect. In conclusion, the str[redacted] induction of c-fos
by glucose and glucoincretins results from Ca(2+) and cAMP signaling pathways addressing both the **CRE** in the promoter and essential response element(s) in the first intron that are unrelated to the transcription arrest site.

L29 ANSWER 2 OF 20 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999349651 MEDLINE
DOCUMENT NUMBER: 99349651
TITLE: Dual intracellular signaling pathways mediated by the human
cannabinoid CB1 receptor.
AUTHOR: Calandra B; Portier M; Kerneis A; Delpech M; Carillon C; Le
Fur G; Ferrara P; Shire D
CORPORATE SOURCE: Sanofi Recherche, Centre de Lab`ege, France.
SOURCE: EUROPEAN JOURNAL OF PHARMACOLOGY, (1999 Jun 25) 374 (3) 445-55.
Journal code: EN6. ISSN: 0014-2999.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
AB It has long been established that the cannabinoid CB1 receptor transduces signals through a pertussis toxin-sensitive Gi/Go inhibitory pathway. Although there have been reports that the cannabinoid CB1 receptor can also mediate an increase in cyclic AMP levels, in most cases the presence of an adenylyl **cyclase** costimulant or the use of very high amounts of agonist was necessary. Here, we present evidence for dual coupling of the cannabinoid CB receptor to the classical pathway and to a pertussis toxin-insensitive adenylyl **cyclase** stimulatory pathway initiated with low quantities of agonist in the absence of any costimulant. Treatment of Chinese hamster ovary (CHO) cells expressing the
cannabinoid CB1 receptor with the cannabinoid CP 55,940, {(-)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hyd roxypropyl) cyclohexan-1-ol} resulted in cyclic AMP accumulation in a dose-response manner, an accumulation blocked by the cannabinoid CB1 receptor-specific antagonist SR 141716A, {N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-me thyl-1H-pyrazole-3-carboxamide hydrochloride}. In CHO cells coexpressing the cannabinoid CB1 receptor and
a cyclic AMP response element (**CRE**)-luciferase **reporter** gene system, CP 55,940 induced luciferase expression by a pathway blocked by the protein kinase A inhibitor N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide hydrochloride (H-89). Under the same conditions the peripheral cannabinoid CB2 receptor proved to be incapable of inducing
cAMP accumulation or luciferase activity. This incapacity allowed us to study the luciferase activation mediated by CB /CB2 chimeric constructs, from which we determined that the first and second internal loop regions of the cannabinoid CB1 receptor were involved in transducing the pathway leading to luciferase gene expression.

L29 ANSWER 3 OF 20 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999121003 MEDLINE
DOCUMENT NUMBER: 99121003
TITLE: Forskolin inhibits cyclin D1 expression in cultured airway smooth-muscle cells.
AUTHOR: Musa N L; Ramakrishnan M; Li J; Kartha S; Liu P; Pestell R G; Hershenson M B
CORPORATE SOURCE: Department of Pediatrics, University of Chicago, Illinois, USA.
CONTRACT NUMBER: HL54685 (NHLBI)
HL56399 (NHLBI)
HL07605 (NHLBI)
+

their combined effe[redacted] on the mutant **CRE** were examine[redacted] Deletion analysis indicated that sequences between -244 and -195 bp were involved in mediating the response to PACAP, with a dramatic reduction in fold-stimulation by PACAP (2.0-fold) of the -195-bp construct, compared with the -244-bp construct (15.8-fold). Constructs containing only upstream alpha-promoter sequences from -517 bp to -98 bp, fused to the heterologous thymidine kinase promoter, exhibited a similar loss of responsiveness to PACAP below -298 bp. Thus, our studies show that, unlike GnRH, PACAP stimulation of alpha-subunit gene transcription in alphaT3-1 cells is less dependent on changes in intracellular calcium concentration; and full transcriptional activation of the alpha-subunit by PACAP requires an intact **CRE**. PACAP responsiveness involves sequences between -244 and -195 bp of the alpha-promoter. These sequences have been implicated also in GnRH-responsiveness and may thus provide a mechanism for coordinated regulation of the alpha-subunit gene by PACAP and GnRH in alphaT3-1 cells.

L29 ANSWER 6 OF 20 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1998133962 MEDLINE
DOCUMENT NUMBER: 98133962
TITLE: Peptidergic activation of transcription and secretion in chromaffin cells. Cis and trans signaling determinants of pituitary adenylyl **cyclase**-activating polypeptide (PACAP).
AUTHOR: Taupenot L; Mahata S K; Wu H; O'Connor D T
CORPORATE SOURCE: Department of Medicine and Center for Molecular Genetics, University of California at San Diego, San Diego, California 92161-9111, USA.
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1998 Feb 15) 101 (4) 863-76.
Journal code: HS7. ISSN: 0021-9738.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199805
ENTRY WEEK: 19980503

AB Pituitary adenylyl **cyclase**-activating polypeptide (PACAP) is a potent endogenous secretagogue for chromaffin cells. Chromogranin A is the major soluble core component in secretory vesicles. Since chromogranin A is secreted along with catecholamines, we asked whether PACAP regulates expression of the chromogranin A gene in PC12 rat chromaffin cells, so as to resynthesize the just-secreted protein, and whether such biosynthetic regulation is coupled mechanistically to catecholamine secretion. PACAP activated the endogenous chromogranin A gene by four- to fivefold. Proportional results (seven- to eightfold activation) were obtained with a transfected 1,200-bp mouse chromogranin A promoter/luciferase **reporter** construct. A series of chromogranin A promoter 5' deletion mutant/luciferase **reporter** constructs narrowed down the PACAP response element to a proximal region containing the cAMP response element (**CRE** box), at (-71 bp)5'-TGACGTAA-3'(-64 bp). Site-directed point mutations of the **CRE** site suppressed PACAP-induced trans-activation of the promoter. Thus, the proximal **CRE** box is entirely necessary for the chromogranin A promoter response to PACAP. Transfer of the **CRE** box to a neutral, heterologous promoter also conferred activation by PACAP, suggesting that the **CRE** domain is also sufficient to mediate the transcriptional response to PACAP. Expression of a dominant-negative mutant (KCREB) of the **CRE**-binding factor CREB markedly diminished trans-activation of the chromogranin A promoter by PACAP. Cotransfection of expression plasmids encoding the protein kinase A inhibitor, or an inactive protein kinase A (PKA) catalytic beta subunit, inhibited both forskolin and PACAP

activation of chromogranin A transcription, revealing that PACAP-induced trans-activation is highly dependent on PKA. By contrast, inhibition of protein kinase C (by chronic exposure to phorbol ester) had no effect on transcriptional activation by PACAP. The potent PACAP/vasoactive intestinal peptide (VIP) type I receptor antagonist PACAP6-38 impaired both chromogranin A transcription or catecholamine secretion triggered by PACAP38, while the PACAP/VIP type II receptor antagonist (p-Chloro-D-Phe6, Leu17)-VIP had little or no ability to antagonize the PACAP38 effect. The agonist VIP was approximately 100- to 1,000-fold less potent than PACAP in stimulating either secretion or transcription. Thus, PACAP-evoked chromogranin A transcription and catecholamine secretion are likely mediated by the PACAP/VIP type I receptor isoform. Although the calcium channel antagonists Zn2+ (100 microM), nifedipine (10 microM), or ruthenium red (10 microM), or the cytosolic calcium chelator BAPTA-AM (50 microM) each strongly impaired PACAP-induced secretion, transcriptional activation of chromogranin A remained unaltered. Therefore, we propose that PACAP signals to chromogranin A transcription through the **CRE** in cis, and through PKA and CREB in trans. By contrast, a pathway involving cytosolic calcium entry through L-type voltage-dependent channels is required for PACAP to evoke catecholamine secretion.

L29 ANSWER 7 OF 20 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1998266969 MEDLINE
DOCUMENT NUMBER: 98266969
TITLE: Transcriptional activation of the macrophage migration-inhibitory factor gene by the corticotropin-releasing factor is mediated by the cyclic adenosine 3',5'- monophosphate responsive element-binding protein CREB in pituitary cells.
AUTHOR: Waeber G; Thompson N; Chautard T; Steinmann M; Nicod P; Pralong F P; Calandra T; Gaillard R C
CORPORATE SOURCE: Department of Internal Medicine B, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.. gwaeber@chuv.hospvd.ch
SOURCE: MOLECULAR ENDOCRINOLOGY, (1998 May) 12 (5) 698-705. Journal code: NGZ. ISSN: 0888-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY WEEK: 19981104

AB Macrophage migration-inhibitory factor (MIF) has recently been identified as a pituitary hormone that functions as a counterregulatory modulator of glucocorticoid action within the immune system. In the anterior pituitary gland, MIF is expressed in TSH- and ACTH-producing cells, and its secretion is induced by CRF. To investigate MIF function and regulation within pituitary cells, we initiated the characterization of the MIF 5'-regulatory region of the gene. The -1033 to +63 bp of the murine MIF promoter was cloned 5' to a luciferase **reporter** gene and transiently transfected into freshly isolated rat anterior pituitary cells. This construct drove high basal transcriptional activity that was further enhanced after stimulation with CRF or with an activator of adenylate **cyclase**. These transcriptional effects were associated with a concomitant rise in ACTH secretion in the transfected cells and by an increase in MIF gene expression as assessed by Northern blot analysis. A cAMP-responsive element (**CRE**) was identified within the MIF promoter region which, once mutated, abolished the cAMP responsiveness of the gene. Using this newly identified **CRE**, DNA-binding activity was detected by gel retardation assay in nuclear extracts prepared from isolated anterior pituitary cells and AtT-20 corticotrope tumor cells. Supershift experiments using antibodies against the **CRE**-binding protein CREB, together with competition assays and the use of recombinant CREB, allowed the detection of CREB-binding activity with the identified MIF **CRE**. These data demonstrate that CREB is the mediator of the CRF-induced MIF gene transcription in pituitary cells through an identified **CRE** in the proximal region of the MIF promoter.

promoter, we report that HHV-6 can efficiently transactivate such genetic elements. Activation of the CD4 promoter occurs in the presence of the viral DNA polymerase inhibitor phosphonoformic acid, which limits expression to the immediate-early and early classes of viral genes. Using deletion mutants and specific CD4 promoter mutants, we identified an ATF/**CRE** binding site located at nucleotides -67 to -60 upstream of the CD4 gene transcription start site that is important for HHV-6 transactivation. The ATF/**CRE** site is also essential for CD4 promoter activation by forskolin, an activator of adenylate **cyclase**. Using electrophoretic mobility shift assays and specific antibodies, we showed that CREB-1 binds specifically to the -79 to -52 region of the CD4 promoter. Last, we have identified two open reading frames (ORFs) of HHV-6, U86 and U89 from the immediate-early locus A, that can transactivate the CD4 promoter in HeLa cells. However, transactivation of the CD4 promoter by ORFs U86 and U89 is independent of the **CRE** element, suggesting that additional HHV-6 ORFs are likely to contribute to CD4 gene activation. Taken together, our results will help to understand the complex interactions occurring between HHV-6 and the CD4 promoter and provide additional information regarding the class of transcription factors involved in the control of CD4 gene expression.

L29 ANSWER 5 OF 20 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998187805 MEDLINE
DOCUMENT NUMBER: 98187805
TITLE: Mechanism of action of pituitary adenylate **cyclase** -activating polypeptide on human glycoprotein hormone alpha-subunit transcription in alphaT3-1 gonadotropes.
AUTHOR: Burrin J M; Aylwin S J; Holdstock J G; Sahye U
CORPORATE SOURCE: Department of Clinical Biochemistry, St. Bartholomew's and the Royal London School of Medicine and Dentistry, United Kingdom.. j.m.burrin@mds.qmw.ac.uk
SOURCE: ENDOCRINOLOGY, (1998 Apr) 139 (4) 1731-7.
Journal code: EGZ. ISSN: 0013-7227.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199806
ENTRY WEEK: 19980603

AB Pituitary adenylate **cyclase** activating polypeptide (PACAP) has been shown to increase glycoprotein hormone alpha-subunit synthesis and release from pituitary cells. We have used alphaT3-1 clonal gonadotropes to investigate the intracellular mechanisms involved in PACAP regulation of alpha-subunit gene transcription; and using deletion, mutation, and heterologous constructs of the alpha-promoter linked to a luciferase **reporter** gene, we have defined DNA sequences responsive to PACAP. Stimulation of alphaT3-1 cells for 24 h with PACAP, GnRH, or vasoactive intestinal peptide (VIP) resulted in a time- and concentration-dependent increase in alpha-promoter transcription at 100 nM for GnRH (17.5-fold, $P < 0.001$), PACAP (12.7-fold, $P < 0.01$), and VIP (4.1-fold, $P < 0.05$). Incubation of alphaT3-1 cells in calcium-depleted medium suggested that the transcriptional response to PACAP was less dependent on changes in intracellular calcium concentration, in contrast to the results seen with GnRH or VIP, where alpha-subunit transcription was significantly reduced. Transfection of an alpha-promoter construct containing a mutant cAMP response element (**CRE**) suggested that the **CRE** region is involved in PACAP and VIP responsiveness, with stimulatory effects on the mutant construct by PACAP (11.1-fold) and VIP (7.6-fold) being significantly ($P < 0.001$) reduced, compared with their stimulatory effects (PACAP: 25.6-fold, VIP: 23.1-fold) on the native alpha-promoter. In the same experiment, the transcriptional response of the mutant **CRE** construct and the native **CRE** construct to GnRH was not significantly different. Both PACAP and VIP enhanced GnRH-stimulated alpha-subunit gene transcription, but this additive effect was lost when

SOURCE: AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY,
(1999 Feb) 20 (2) 352-8.
Journal code: AOB. ISSN: 1044-1549.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199905
ENTRY WEEK: 19990502

AB Accumulation of intracellular cyclic adenosine monophosphate (cAMP) has been shown to inhibit the growth of cultured airway smooth-muscle cells, but the precise mechanism underlying the antimitogenic action of cAMP in these cells is unknown. We examined the effects of forskolin, an activator of adenylate **cyclase**, on DNA synthesis, cyclin D1 expression, and cAMP response element-binding protein (CREB) phosphorylation and DNA binding in bovine tracheal myocytes. DNA synthesis was assessed by measurement of [3H]thymidine incorporation. Cyclin D1 protein abundance and CREB phosphorylation were assessed by immunoblotting. Cyclin D1 promoter transcriptional activation was determined by measurement of luciferase activity in cells transiently cotransfected with complementary DNAs encoding the full-length cyclin D1 promoter subcloned into a luciferase **reporter** and beta-galactosidase (to normalize for transfection efficiency). The binding of nuclear proteins to the cyclin D1 promoter cAMP response element (**CRE**) was determined by electrophoretic mobility shift assay. We found that forskolin attenuated platelet-derived growth factor-induced DNA synthesis in a concentration-dependent manner. In addition, forskolin pretreatment decreased both cyclin D1 promoter activity and protein levels. Forskolin treatment induced the phosphorylation of CREB and increased the binding of nuclear protein to the cyclin D1 promoter **CRE**. Finally, addition of an antibody against CREB1 induced supershift of at least one protein-DNA complex. Together, these data suggest that cAMP suppresses cyclin D1 gene expression via phosphorylation and transactivation of CREB. Further studies are needed to determine whether this is the primary mechanism of cAMP-induced growth inhibition, or whether additional pathways are also involved.

L29 ANSWER 4 OF 20 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998440543 MEDLINE
DOCUMENT NUMBER: 98440543
TITLE: CD4 promoter transactivation by human herpesvirus 6.
AUTHOR: Flamand L; Romerio F; Reitz M S; Gallo R C
CORPORATE SOURCE: Laboratory of Virology, Rheumatology and Immunology Research Center, Centre de Recherche du CHUL and Laval University, Sainte-Foy, Quebec, Canada..
louis.flamand@crchul.ulaval.ca
CONTRACT NUMBER: RO3 AI41854-02 (NIAID)
SOURCE: JOURNAL OF VIROLOGY, (1998 Nov) 72 (11) 8797-805.
Journal code: KCV. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199901
ENTRY WEEK: 19990104

AB The observation that human herpesvirus 6 (HHV-6) can induce CD4 gene transcription and expression in CD4(-) cells was reported several years ago (P. Lusso, A. De Maria, M. Malnati, F. Lori, S. E. DeRocco, M. Baseler, and R. C. Gallo, Nature 349:533-535, 1991) and subsequently confirmed (P. Lusso, M. S. Malnati, A. Garzino-Demo, R. W. Crowley, E. O. Long, and R. C. Gallo, Nature 362:458-462, 1993; G. Furlini, M. Vignoli, E. Ramazzotti, M. C. Re, G. Visani, and M. LaPlaca, Blood 87:4737-4745, 1996). Our objective was to identify the mechanisms underlying such phenomena. Using **reporter** gene constructs driven by the CD4

L29 ANSWER 8 OF 20 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1998161536 MEDLINE
DOCUMENT NUMBER: 98161536
TITLE: Pituitary adenylate **cyclase**-activating polypeptide stimulates proenkephalin gene transcription through AP1- and CREB-dependent mechanisms.
AUTHOR: Monnier D; Loeffler J P
CORPORATE SOURCE: Institut de Physiologie et de Chimie Biologique, URA 1446 CNRS Neurophysiologie et Neurobiologie des Syst`emes Endocrines, Strasbourg, France.
SOURCE: DNA AND CELL BIOLOGY, (1998 Feb) 17 (2) 151-9. Journal code: AF9. ISSN: 1044-5498.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY WEEK: 19980503
AB The effects of the pituitary adenylase cyclate-activating peptides (PACAP) 27 and 38 on proenkephalin (PENK) gene transcription were examined in PC12 (rat pheochromocytoma) cells using transient transfection assays. Both ligands stimulated PENK gene transcription in a dose-dependent manner, with an apparent ED50 close to 5 x 10(-11) M. Inactivation of cAMP dependent-protein kinase (PKA) with a dominant inhibitory mutant strongly reduced PACAP-stimulated PENK transcription. Using **reporter** genes driven by either the minimal TPA-responsive element (TRE: TGACTCA) or cAMP-responsive element (**CRE**: TGACGTCA), we showed that the two PACAPs activate transcription through both regulatory sequences. These effects could result from direct post-translational activation of Jun and CREB, as shown using GAL4-Jun or GAL4-CREB fusion proteins. Expression of a dominant inhibitory mutant of CREB decreased by 60% the response to PACAP, suggesting that CREB is implicated in PENK transactivation. Similarly, expression of c-fos antisense RNA reduced by 80% the stimulatory effects of PACAP. Taken together, these results indicate that PACAP stimulates PENK transcription by members of both the AP1 and the CREB families. However, AP1 by itself is not sufficient to increase PENK transcription, as insulin-like growth factor 1 (IGF1), which stimulates AP1 activity but not cAMP production, is unable to stimulate PENK transcription. These results indicate a cooperative effect of AP1 and CREB on PENK transcription.

L29 ANSWER 9 OF 20 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 1998368775 MEDLINE
DOCUMENT NUMBER: 98368775
TITLE: Parathyroid hormone responses of cyclic AMP-, serum- and phorbol ester-responsive **reporter** genes in osteoblast-like UMR-106 cells.
AUTHOR: Fluhmann B; Zimmermann U; Muff R; Bilbe G; Fischer J A; Born W
CORPORATE SOURCE: Research Laboratory for Calcium Metabolism, Department of Orthopedic Surgery, Zurich, Switzerland.
SOURCE: MOLECULAR AND CELLULAR ENDOCRINOLOGY, (1998 Apr 30) 139 (1-2) 89-98. Journal code: E69. ISSN: 0303-7207.
PUB. COUNTRY: Ireland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
AB Parathyroid hormone (PTH) and PTH-related protein interact with a G protein-coupled receptor linked to the activation of adenylyl **cyclase** and phospholipase C signaling pathways. Regulation by PTH of the expression of three distinct, stably transfected luciferase **reporter** genes responsive to cAMP (**CRE**-luc), serum

(SRE-luc) and phorbol ester (TRE-luc) has been studied in rat osteoblast-like UMR-106 cells. Maximal 43-fold induction of **CRE** -luc expression occurred in response to 100 nM rat (r)PTH(1-34) (EC50=0.44 nM), but SRE-luc and TRE-luc remained unaffected. Maximal 2.8- and 3.4-fold inductions of SRE-luc by 10 ng/ml EGF and 100 nM phorbol ester (PMA) were suppressed with 100 nM rPTH(1-34) (IC50=0.04 and 0.15 nM, respectively). Similarly, 7.3-fold induction of TRE-luc by 100 nM PMA was inhibited to 50% with 100 nM rPTH(1-34) (IC50=0.5 nM). Activation of mitogen-activated protein kinase by EGF and PMA was also suppressed by rPTH(1-34). 1 mM 8-Br-cAMP and 0.1 mM forskolin mimicked all the effects of rPTH(1-34). In conclusion, the regulation of target genes by PTH in osteoblast-like UMR-106 cells is mediated by the activation of the cAMP/protein kinase A signaling pathway.

L29 ANSWER 10 OF 20 MEDLINE
ACCESSION NUMBER: 1999126958 MEDLINE
DOCUMENT NUMBER: 99126958
TITLE: Cloning and functional characterization of the human VIP1/PACAP receptor promoter.
AUTHOR: Couvineau A; Maoret J J; Rouyer-Fessard C; Carrero I; Laburthe M
CORPORATE SOURCE: Laboratoire de Neuroendocrinologie et Biologie Cellulaire Digestives, Institut National de la Sante et de la Recherche Medicale, INSERM U-410, Faculte de Medecine Xavier Bichat, Paris, France.. coucou@bichat.inserm.fr
SOURCE: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1998 Dec 11) 865 59-63.
Journal code: 5NM. ISSN: 0077-8923.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199904
ENTRY WEEK: 19990403
AB The 5'-flanking region (1.5 kb) of the gene coding for the human VIP1/PACAP receptor was isolated, sequenced, and characterized. Transient expression of constructs containing sequentially deleted 5'-flanking sequences of the VIP1/PACAP receptor fused to a luciferase **reporter** gene showed that this sequence was active as a promoter in the intestinal cancer cell line, HT-29, expressing endogenous VIP1/PACAP receptor. The shortest DNA fragment with significant promoter activity encompassed the region from -205 to +76 bp. Deletion of a CCAAT-box sequence in the construction corresponding to -173 to +76 bp dramatically reduced the promoter activity. The promoter -205 to +76 bp has a housekeeping gene structure without TATA-box. It contains GC-rich regions characterized by potential Sp1 and AP2 sites and some potential regulatory elements, such as **CRE** and ATF, and a CCAAT-box sequence (-182 to -178) crucial for gene transcription.

L29 ANSWER 11 OF 20 MEDLINE
ACCESSION NUMBER: 1999126953 MEDLINE
DOCUMENT NUMBER: 99126953
TITLE: Cis-regulatory elements controlling basal and inducible VIP
gene transcription.
AUTHOR: Hahm S H; Eiden L E
CORPORATE SOURCE: Section on Molecular Neuroscience, National Institutes of Health, Bethesda, Maryland 20892, USA.
SOURCE: ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1998 Dec 11) 865 10-26. Ref: 48
Journal code: 5NM. ISSN: 0077-8923.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199904

ENTRY WEEK: 1990

AB The cis-acting elements of the VIP gene important for basal and stimulated transcription have been studied by transfection of VIP-**reporter** gene constructs into distinct human neuroblastoma cell lines in which VIP transcription is constitutively high, or can be induced to high levels by protein kinase stimulation. The 5.2 kb flanking sequence of the VIP gene conferring correct basal and inducible VIP gene expression onto a **reporter** gene in these cell lines was systematically deleted to define its minimal components. A 425-bp fragment (-4656 to -4231) fused to the proximal 1.55 kb of the VIP promoter-enhancer was absolutely required for cell-specific basal and inducible transcription. Four additional components of the VIP gene were required for full cell-specific expression driven by the 425 bp TSE (region A). Sequences from -1.55 to -1.37 (region B), -1.37 to -1.28 (region C), -1.28 to -.094 (region D), and the **CRE**-containing proximal 94 bp (region E) were deleted in various combinations to demonstrate the specific contributions of each region to correct basal and inducible VIP gene expression. Deletion of region B, or mutational inactivation of the **CRE** in region E, resulted in constructs with low transcriptional activity in VIP-expressing cell lines.

Deletion of regions B and C together resulted in a gain of transcriptional activity, but without cell specificity. All five domains of the VIP gene were also required for cell-specific induction of VIP gene expression with phorbol ester. Gelshift analysis of putative regulatory sequences in regions A-D suggests that both ubiquitous and neuron-specific trans-acting proteins participate in VIP gene regulation.

L29 ANSWER 12 OF 20 MEDLINE DUPLICATE 10

ACCESSION NUMBER: 96198775 MEDLINE
DOCUMENT NUMBER: 96198775
TITLE: Induction of chinook salmon growth hormone promoter activity by the adenosine 3',5'-monophosphate (cAMP)-dependent pathway involves two cAMP-response elements with the CGTCA motif and the pituitary-specific transcription factor Pit-1.
AUTHOR: Wong A O; Le Drean Y; Liu D; Hu Z Z; Du S J; Hew C L
CORPORATE SOURCE: Hospital for Sick Children, Department of Clinical Biochemistry, University of Toronto, Ontario, Canada.
SOURCE: ENDOCRINOLOGY, (1996 May) 137 (5) 1775-84.
Journal code: EGZ. ISSN: 0013-7227.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 199608

AB In this study, the functional role of two cAMP-response elements (**CRE**) in the promoter of the chinook salmon GH gene and their interactions with the transcription factor Pit-1 in regulating GH gene expression were examined. A chimeric construct of the chloramphenicol acetyltransferase (CAT) **reporter** gene with the **CRE**-containing GH promoter (pGH.CAT) was transiently transfected into primary cultures of rainbow trout pituitary cells. The expression of CAT activity was stimulated by an adenylate **cyclase** activator forskolin as well as a membrane-permeant cAMP analog 8-bromo-cAMP. Furthermore, these stimulatory responses were inhibited by a protein kinase A inhibitor H89, suggesting that these CREs are functionally coupled to the adenylate **cyclase**-cAMP-protein kinase A cascade. This hypothesis is supported by parallel studies using GH4ZR7 cells, a rat pituitary cell line stably transfected with dopamine D2 receptors. In this cell line, D2 receptor activation is known to inhibit adenylate **cyclase**

activity and cAMP synthesis. Stimulation with a nonselective dopamine agonist, apomorphine, or a D2-specific agonist, Ly171555, suppressed the expression of pGH.CAT in GH4ZR7 cells, and this inhibition was blocked by simultaneous treatment with forskolin. These results indicate that inhibition of the cAMP-dependent pathway reduces the basal promoter activity of the **CRE**-containing pGH.CAT. The functionality of these CREs was further confirmed by deletion analysis and site-specific mutagenesis. In trout pituitary cells, the cAMP inducibility of pGH.CAT was inhibited after deleting the **CRE**-containing sequence from the GH promoter. When the **CRE**-containing sequence was cloned into a CAT construct with a viral thymidine kinase promoter, a significant elevation of cAMP inducibility was observed. This stimulatory response, however, was abolished by mutating the core sequence, CGTCA, in these CREs, suggesting that these cis-acting elements confer cAMP inducibility to the salmon GH gene. The interactions between CREs and the transcription factor Pit-1 in mediating GH gene expression were also examined. In HeLa cells, a human cervical cancer cell line deficient in Pit-1, both basal and cAMP-induced expression of pGH.CAT were apparent only with the cotransfection of a Pit-1 expression vector. These results taken together indicate that the two CREs in the chinook salmon GH gene are functionally associated with the cAMP-dependent pathway and that their promoter activity is dependent on the presence of Pit-1

L29 ANSWER 13 OF 20 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 95255611 MEDLINE
DOCUMENT NUMBER: 95255611
TITLE: Nitric oxide and cGMP analogs activate transcription from AP-1-responsive promoters in mammalian cells.
AUTHOR: Pilz R B; Suhasini M; Idriss S; Meinkoth J L; Boss G R
CORPORATE SOURCE: Department of Medicine, University of California, San Diego, La Jolla 92093-0652, USA..
CONTRACT NUMBER: CA01538 (NCI)
DK45696 (NIDDK)
GM49360 (NIGMS)
SOURCE: FASEB JOURNAL, (1995 Apr) 9 (7) 552-8.
Journal code: FAS. ISSN: 0892-6638.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199508

AB Nitric oxide (NO) increases cytosolic guanylate **cyclase** activity and thereby activates the cGMP signal transduction pathway. The cAMP and Ca2+/phospholipid signal transduction pathways activate transcription factors that bind to the cAMP response element (**CRE**) and phorbol ester response element (TRE), respectively. Little is known about transcriptional regulation of gene expression by NO/cGMP. In transient and stable transfection experiments and in microinjection studies we found that three different NO-releasing agents and two membrane-permeable cGMP analogs activated TRE-regulated but not **CRE**-regulated **reporter** genes in rodent fibroblast and epithelial cell lines. Activation of TRE-regulated genes by NO-releasing agents and cGMP analogs appeared to be mediated by the AP-1 (Jun/Fos) transcription factor complex because we observed increased DNA binding of AP-1 and increased junB and c-fos mRNA in cells treated with these agents. The mechanism of gene activation by NO/cGMP was distinct from that used by phorbol esters and cAMP because it was not associated with c-jun mRNA induction and was not observed with **CRE**-containing promoters.

L29 ANSWER 14 OF 20 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 95050735 MEDLINE
DOCUMENT NUMBER: 95050735
TITLE: Regulation of cAMP-mediated gene transcription by wild type and mutated G-protein alpha subunits. Inhibition of

adenylate **cyclase** activity by muscarinic receptor-activated and constitutively activated G(o) alpha.
AUTHOR: Migeon J C; Thomas S L; Nathanson N M
CORPORATE SOURCE: Department of Pharmacology, University of Washington, Seattle 98195.
CONTRACT NUMBER: GM07108 (NIGMS)
GM07750 (NIGMS)
NS07332 (NINDS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Nov 18) 269 (46) 29146-52.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199502
AB We have used a luciferase **reporter** gene under the transcriptional control of a cAMP response element (**CRE**) to monitor the effects of G-protein alpha subunits on cAMP-regulated gene expression and to examine muscarinic acetylcholine receptor (mAChR) functional coupling to G-proteins. Expression in JEG-3 cells of a mutationally activated Gi alpha-2 in which glutamine 205 is replaced with leucine (Q205L) decreased forskolin-stimulated expression from the **CRE**-luciferase gene by up to 75%. Similarly, mutation of glycine 43 (corresponding to glycine 12 in p21ras) to valine decreased forskolin-stimulated expression from the **CRE**-luciferase gene by a maximum of 50%, indicating that this mutation activates the G-protein and is potentially oncogenic. Transfection of the activated Q205L G(o) alpha subunit decreased forskolin stimulation of **CRE**-luciferase expression. Transfected wild type G(o) alpha was also able to couple the m4 mAChR receptor to inhibition of AC. The amino-terminal myristoylation site was removed from wild type Gi alpha-2 and Q205L Gi alpha-2 by changing glycine 2 to alanine (G2A). Gi alpha-2 with the G2A and Q205L mutations was unable to decrease forskolin stimulation of **CRE** -mediated luciferase activity. Furthermore, G2A Gi alpha-2 was unable to couple the m4 mAChR to inhibition of AC. Thus, myristoylation is required both for the function of constitutively active Q205L Gi alpha-2 and for receptor-mediated activation of wild type Gi alpha-2.

L29 ANSWER 15 OF 20 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 94128108 MEDLINE
DOCUMENT NUMBER: 94128108
TITLE: Functional coupling of human adenosine receptors to a ligand-dependent **reporter** gene system.
AUTHOR: Castanon M J; Spevak W
CORPORATE SOURCE: Ernst Boehringer Institute, Vienna, Austria.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1994 Jan 28) 198 (2) 626-31.
Journal code: 9Y8. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199405
AB We have stably transfected CHO cells that have integrated in their genome a **reporter** gene under the control of promoter sequences containing several copies of the cAMP response element, **CRE**, with different human adenosine receptors: A1, A2a and A2b. The new cell lines responded to the addition of known adenosine agonists and antagonists with changes in the expression of the **reporter** gene. The activity of the **reporter** gene can be easily monitored by bioluminescence. Although adenosine receptors are divergently coupled to adenylate **cyclase**, A1 receptors inhibit whereas A2 stimulate, changes in gene expression faithfully reflected the negative and positive coupling of the receptors. We have used the system to examine the pharmacological profile of the human receptors expressed in CHO cells.

L29 ANSWER 16 OF 20 MEDLINE DUPLICATE 14

ACCESSION NUMBER: 95148[redacted] MEDLINE
DOCUMENT NUMBER: 95148057
TITLE: Pituitary adenyl **cyclase**-activating peptide: a hypophysiotropic factor that stimulates proopiomelanocortin gene transcription, and proopiomelanocortin-derived peptide secretion in corticotropic cells.
AUTHOR: Boutillier A L; Monnier D; Koch B; Loeffler J P
CORPORATE SOURCE: Institut de Physiologie et de Chimie Biologie, URA 1446 CNRS Neurophysiologie et Neurobiologie des Syst`emes Endocrines, Strasbourg, France.
SOURCE: NEUROENDOCRINOLOGY, (1994 Nov) 60 (5) 493-502.
Journal code: NY8. ISSN: 0028-3835.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
AB The biological effects of pituitary adenylate **cyclase**-activating peptide (PACAP) 27 and 38 on peptide secretion and gene regulation were studied in the mouse corticotrope-derived cell line AtT20. Treatment of these cells with PACAP 27/38 led to a dose-dependent increase in cAMP content and ACTH accumulation in the medium with an apparent ED50 value close to 10(-9) M. The genomic effects of PACAP were first investigated by using a **reporter** gene containing a cAMP responsive element (**CRE**: TGACGTCA) PACAP 27/38 stimulate transcription from this construction and the effect is further increased when cells are cotreated with the phosphodiesterase inhibitor rolipram. Furthermore, we show by measuring nuclear heterologous proopiomelanocortin (POMC) RNA levels or by using a **reporter** gene containing the POMC promoter region, that PACAP stimulates POMC transcription. This transcriptional stimulation is mediated by the cAMP-dependent protein kinase (PKA) since genetic inactivation of PKA by a dominant inhibitory mutant of this enzyme completely abolished the effect of PACAP on POMC transcription. Finally, we show that the transcriptional stimulation of POMC by PACAP is repressed by the glucocorticoid receptor agonist dexamethasone. Taken together, these data suggest that PACAP is a hypophysiotropic hormone that exert similar if not identical functions as corticotropin-releasing hormone (CRH) on corticotrope cells.

L29 ANSWER 17 OF 20 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 94043130 MEDLINE
DOCUMENT NUMBER: 94043130
TITLE: An AP-1-like factor and the pituitary-specific factor Pit-1 are both necessary to mediate hormonal induction of human thyrotropin beta gene expression.
AUTHOR: Kim M K; McClaskey J H; Bodenner D L; Weintraub B D
CORPORATE SOURCE: Molecular and Cellular Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Nov 5) 268 (31) 23366-75.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199402
AB The human thyrotropin beta (hTSH beta) gene is inducible by various agents including thyrotropin-releasing hormone, phorbol esters, or the adenylyl **cyclase** activator forskolin. In this study, we have characterized the functional properties of the TGGGTCA motif at -1/+6 of the hTSH beta gene that is similar to the consensus phorbol ester response element (TRE)

or the consensus cyclic AMP response element (**CRE**). We suggest that both protein kinases C and A as well as TRH share a common mediator which recognizes the TGGGTCA element in activating the hTSH beta promoter.

Following stimulation by phorbol esters, forskolin, or TRH, the TGGGTCA-specific factor acts together with the pituitary-specific transcription factor Pit-1 (or GHF-1) bound to upstream sequences at -128 to -61 to mediate the induction of the hTSH beta promoter. The induction requires that both factors bind to their own binding sites, but Pit-1 neither increases the binding of the TGGGTCA-specific factor to its target sequences nor associates with this factor to form a heterodimer. The TGGGTCA-specific factor is present in three cell lines tested and is composed of protein(s) immunologically related to c-Jun and c-Fos but not to the **CRE**-binding protein, CREB. By using the hTSH beta **reporter** plasmids in which the TGGGTCA element is converted to consensus TRE or **CRE** motifs, we found that, within the context of the hTSH beta promoter, the TGGGTCA element is a more potent TRE or **CRE** than the consensus TRE or **CRE** sequences. Based upon the results of this study, we propose a model in which the TGGGTCA-specific AP-1-like factor functionally cooperates with the tissue-specific factor Pit-1 to activate the hTSH beta gene.

L29 ANSWER 18 OF 20 MEDLINE DUPLICATE 16

ACCESSION NUMBER: 93366587 MEDLINE
DOCUMENT NUMBER: 93366587
TITLE: Cyclic AMP mediated gene expression in bovine corneal endothelial cells.
AUTHOR: Gonzalez G A; Feldman S T
CORPORATE SOURCE: Department of Ophthalmology, University of California/San Diego, La Jolla 92093.
CONTRACT NUMBER: AG000353 (NIA)
SOURCE: INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, (1993 Sep) 34 (10) 2970-5.
Journal code: GWI. ISSN: 0146-0404.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312

AB PURPOSE. Agents that increase intracellular levels of cAMP mediate gene expression associated with cellular morphology, growth, and/or differentiation via the cAMP response element (**CRE**). The cAMP element binding protein (CREB) is a transcriptional activator that binds and stimulates gene expression from the **CRE** in the promoters of cAMP responsive genes. This study was designed to characterize the cyclic AMP (cAMP) transcription apparatus in bovine corneal endothelial cells (BCE). METHODS. **CRE** transcriptional activity was determined by transient transfection assays using the **CRE**-chloramphenicol acetyl transferase gene (**CRE**-CAT) fusion **reporter** construct. Western blot analyses were performed to determine whether CREB was present in BCE. Mobility shift DNA-binding assay using gel electrophoresis and DNase I protection assays were performed to exclude the possibility of other **CRE**-binding factors. RESULTS. The authors identified the transcription factor, CREB, in nuclear extracts from BCE by Western blot analysis and showed that its DNA-binding characteristics are identical to the previously characterized CREB protein by DNase I protection and mobility shift DNA-binding studies. Transient transfection studies using the **CRE**-CAT **reporter** constructs revealed that the beta-adrenergic receptor agonist, isoproterenol, stimulates gene expression to levels similar to those induced by forskolin, a direct activator of adenylate **cyclase** (6.0- and 7.2-fold, respectively). CONCLUSIONS. The results suggest that agents that modulate receptors coupled to adenylate **cyclase** may effect the corneal endothelium by altering gene expression through the second messenger, cAMP.

L29 ANSWER 19 OF 20 MEDLINE

ACCESSION NUMBER: 9319[redacted] MEDLINE
DOCUMENT NUMBER: 93195864
TITLE: Functional testing of human dopamine D1 and D5 receptors expressed in stable cAMP-responsive luciferase **reporter** cell lines.
AUTHOR: Himmler A; Stratowa C; Czernilofsky A P
CORPORATE SOURCE: Ernst Boehringer Institut, Bender + Co. GmbH, Vienna, Austria..
SOURCE: JOURNAL OF RECEPTOR RESEARCH, (1993) 13 (1-4) 79-94.
Journal code: JWM. ISSN: 0197-5110.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199306

AB A large number of G-protein coupled receptors are known to modulate adenylyl **cyclase** activity. In order to find new compounds modulating the activity of specific receptor subtypes we developed a cellular screening system that measures the biological activity of drugs acting on receptors rather than merely their binding characteristics. The activity of the receptor coupling to the cAMP signal transduction pathway was measured via transcriptional activation of a **reporter** gene. A chinese hamster ovary cell line was stably transformed with a **reporter** plasmid containing the firefly luciferase gene under the transcriptional control of multiple cAMP responsive elements (**CRE**). This **CRE reporter** cell line exhibited 20 to 30-fold induction of luciferase activity upon stimulation of adenylyl **cyclase** with forskolin, but did not respond to dopamine agonists. Stable test cell lines were developed by transfecting **reporter** cell lines with human dopamine D1 and D5 receptor genes, respectively. Treatment of these test cell lines with dopamine receptor agonists and antagonists modulated the luciferase expression in a dose-dependent manner. The rank of potency of dopamine receptor agonists and antagonists was in agreement with reported data obtained from binding studies. The non-isotopic assay can be performed in microtiter plate format and is far less work intensive than the determination of adenylyl **cyclase** activity by direct cAMP measurement. This technology could also be utilized for discovery of new classes of compounds, e.g. allosteric effectors or non competitive ligands.

L29 ANSWER 20 OF 20 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 92017868 MEDLINE
DOCUMENT NUMBER: 92017868
TITLE: Regulation of the alpha inhibin gene by cyclic adenosine 3',5'-monophosphate after transfection into rat granulosa cells.
AUTHOR: Pei L; Dodson R; Schoderbek W E; Maurer R A; Mayo K E
CORPORATE SOURCE: Department of Biochemistry, Northwestern University, Evanston, Illinois 60208.
CONTRACT NUMBER: HD-27491 (NICHD)
HD-21921 (NICHD)
DK-36407 (NIDDK)
SOURCE: MOLECULAR ENDOCRINOLOGY, (1991 Apr) 5 (4) 521-34.
Journal code: NGZ. ISSN: 0888-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-S56581; GENBANK-S56855; GENBANK-S85365; GENBANK-S85371; GENBANK-S85222; GENBANK-S85224; GENBANK-S79268; GENBANK-S79270; GENBANK-S79277; GENBANK-M64566
ENTRY MONTH: 199201

AB Inhibin gene expression in the ovary is stimulated by FSH, which uses cAMP as an intracellular second messenger. To examine further the transcriptional regulation of the alpha inhibin gene by FSH and cAMP, we have isolated and characterized a genomic clone that contains the entire rat alpha inhibin gene. Sequence analysis of the alpha inhibin promoter

region revealed several potential cAMP response elements (CREs) and transcription factor AP2-binding sites that might mediate cAMP regulation.

To determine the functional importance of these sequences, fusion genes including the alpha inhibin 5' flanking region linked to a luciferase **reporter** gene were transiently transfected into primary granulosa cells isolated from immature rats. These fusion genes were both expressed and regulated by the adenylyl **cyclase** activator forskolin in transfected granulosa cells. Analysis of a series of 5' deletion mutants indicated that a construct containing as little as 170 basepairs up-stream of the alpha inhibin start site, which includes a single imperfect **CRE** and no AP2 sites, was regulated by forskolin. DNAse footprinting was used to demonstrate that bacterially expressed **CRE**-binding protein (CREB) binds to this **CRE** located 122 basepairs up-stream of the alpha inhibin gene transcriptional start site. To investigate further the role of this **CRE** in alpha inhibin gene expression, site-specific mutagenesis of the **CRE** was performed. The alpha inhibin promoter containing a mutated **CRE** was not regulated by forskolin in granulosa cells and did not bind the CREB protein. Interestingly, mutation of the **CRE** also substantially reduced basal expression of the alpha inhibin promoter. Lastly, a gel mobility shift assay was used to examine **CRE**-binding proteins from granulosa cell extracts. Granulosa cells contain a protein that specifically interacts with **CRE**-containing oligonucleotides or with the alpha inhibin promoter and that is recognized by antibodies against the CREB protein. Our results suggest that CREB or related transcription factors play an important role in both basal and cAMP-regulated expression of the alpha inhibin gene in ovarian granulosa cells.

```
=> s pka

L30      13729 PKA

=> d his

     (FILE 'HOME' ENTERED AT 14:41:49 ON 02 MAY 2000)

     FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000
L1         128 S CYCLASE AND CRE
L2        2756 S (FEEDBACK LOOP)
L3           1 S L1 AND L2
L4        3561 S CREB
L5          15 S L2 AND L4
L6           9 DUP REM L5 (6 DUPLICATES REMOVED)
L7       35925 S REPORTER
L8           2 S L6 AND L7
L9           4 S AUTOPOSITIVE
L10         30 S AUTO-FEEDBACK
L11      77963 S AMPLIFICATION
L12     131206 S AMPLIF?
L13        276 S REPORTER ASSAY
L14       2788 S L2 OR L9 OR L10
L15          0 S L13 AND L14
L16         47 S L14 AND REPORTER
L17         28 DUP REM L16 (19 DUPLICATES REMOVED)
L18         28 S L14 AND L17
L19          3 S L17 AND L12
L20       3124 S REPORTER CONSTRUCT?
L21      77963 S L11
L22         77 S L11 AND L20
L23          4 S L22 AND L14
L24          2 DUP REM L23 (2 DUPLICATES REMOVED)
L25          1 S L1 AND L12
L26         37 S L1 AND L7
```

L27 0 S L26 AND L6
L28 0 S L26 AND L14
L29 20 DUP REM L26 (17 DUPLICATES REMOVED)
L30 13729 S PKA

=> s l30 and l14

L31 16 L30 AND L14

=> s l30 and l14

L32 16 L30 AND L14

=> dup rem l32

PROCESSING COMPLETED FOR L32
L33 9 DUP REM L32 (7 DUPLICATES REMOVED)

=> l33 and l7

L33 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l33 and l7

L34 0 L33 AND L7

=> d ibib abs l33

L33 ANSWER 1 OF 9 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999214556 MEDLINE
DOCUMENT NUMBER: 99214556
TITLE: Inhibition of phospholipase A2-mediated arachidonic acid release by cyclic AMP defines a negative **feedback loop** for P2Y receptor activation in Madin-Darby canine kidney D1 cells.
AUTHOR: Xing M; Post S; Ostrom R S; Samardzija M; Insel P A
CORPORATE SOURCE: Department of Pharmacology, University of California, San Diego, La Jolla, California 92093, USA.
CONTRACT NUMBER: GM31987 (NIGMS)
HL35847 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Apr 9) 274 (15) 10035-8.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199907
ENTRY WEEK: 19990702
AB In Madin-Darby canine kidney D1 cells extracellular nucleotides activate P2Y receptors that couple to several signal transduction pathways, including stimulation of multiple phospholipases and adenylyl cyclase. For one class of P2Y receptors, P2Y2 receptors, this stimulation of adenylyl cyclase and increase in cAMP occurs via the conversion of phospholipase A2 (PLA2)-generated arachidonic acid (AA) to prostaglandins (e.g. PGE2). These prostaglandins then stimulate adenylyl cyclase activity, presumably via activation of prostanoid receptors. In the current study we show that agents that increase cellular cAMP levels (including PGE2, forskolin, and the beta-adrenergic agonist isoproterenol) can inhibit P2Y receptor-promoted AA release. The protein kinase A (**PKA**) inhibitor H89 blocks this effect, suggesting that this feedback inhibition occurs via activation of **PKA**. Studies with PGE2 indicate that

inhibition of AA release is attributable to inhibition of mitogen-activated protein kinase activity and in turn of P2Y receptor stimulated PLA2 activity. Although cAMP/**PKA**-mediated inhibition occurs for P2Y receptor-promoted AA release, we did not find such inhibition for epinephrine (alpha1-adrenergic) or bradykinin-mediated AA release. Taken together, these results indicate that negative feedback regulation via cAMP/**PKA**-mediated inhibition of mitogen-activated protein kinase occurs for some, but not all, classes of receptors that promote PLA2 activation and AA release. We speculate that receptor-selective feedback inhibition occurs because PLA2 activation by different receptors in Madin-Darby canine kidney D1 cells involves the utilization of different signaling components that are differentially sensitive to increases in cAMP or, alternatively, because of compartmentation of signaling components.

=> d ibib abs 1-9

L34 HAS NO ANSWERS
'1-9 ' IS NOT A VALID SEARCH STATUS KEYWORD
Search status keywords:
NONE ---- Display only the number of postings.
STATUS -- Display statistics of the search.
ENTER SEARCH STATUS OPTION (NONE), STATUS, OR ?:end

=> d ibib abs 1-9 l33

L33 ANSWER 1 OF 9 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999214556 MEDLINE
DOCUMENT NUMBER: 99214556
TITLE: Inhibition of phospholipase A2-mediated arachidonic acid release by cyclic AMP defines a negative **feedback loop** for P2Y receptor activation in Madin-Darby canine kidney D1 cells.
AUTHOR: Xing M; Post S; Ostrom R S; Samardzija M; Insel P A
CORPORATE SOURCE: Department of Pharmacology, University of California, San Diego, La Jolla, California 92093, USA.
CONTRACT NUMBER: GM31987 (NIGMS)
HL35847 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Apr 9) 274 (15) 10035-8.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199907
ENTRY WEEK: 19990702
AB In Madin-Darby canine kidney D1 cells extracellular nucleotides activate P2Y receptors that couple to several signal transduction pathways, including stimulation of multiple phospholipases and adenylyl cyclase. For one class of P2Y receptors, P2Y2 receptors, this stimulation of adenylyl cyclase and increase in cAMP occurs via the conversion of phospholipase A2 (PLA2)-generated arachidonic acid (AA) to prostaglandins (e.g. PGE2). These prostaglandins then stimulate adenylyl cyclase activity, presumably via activation of prostanoid receptors. In the current study we show that agents that increase cellular cAMP levels (including PGE2, forskolin, and the beta-adrenergic agonist isoproterenol) can inhibit P2Y receptor-promoted AA release. The protein kinase A (**PKA**) inhibitor H89 blocks this effect, suggesting that this feedback inhibition occurs via activation of **PKA**. Studies with PGE2 indicate that inhibition of AA release is attributable to inhibition of mitogen-activated protein kinase activity and in turn of P2Y receptor stimulated PLA2 activity. Although cAMP/**PKA**-mediated inhibition

occurs for P2Y receptor-promoted AA release, we did not find such inhibition for epinephrine (alpha1-adrenergic) or bradykinin-mediated AA release. Taken together, these results indicate that negative feedback regulation via cAMP/**PKA**-mediated inhibition of mitogen-activated protein kinase occurs for some, but not all, classes of receptors that promote PLA2 activation and AA release. We speculate that receptor-selective feedback inhibition occurs because PLA2 activation by different receptors in Madin-Darby canine kidney D1 cells involves the utilization of different signaling components that are differentially sensitive to increases in cAMP or, alternatively, because of compartmentation of signaling components.

L33 ANSWER 2 OF 9 MEDLINE
ACCESSION NUMBER: 1998400153 MEDLINE
DOCUMENT NUMBER: 98400153
TITLE: Role of **PKA** in the timing of developmental events in Dictyostelium cells.
AUTHOR: Loomis W F
CORPORATE SOURCE: Center for Molecular Genetics, Department of Biology, University of California San Diego, La Jolla, California 92093, USA.. wloomis@UCSD.edu
CONTRACT NUMBER: HD30892 (NICHD)
SOURCE: MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, (1998 Sep) 62 (3) 684-94. Ref: 120
Journal code: CS0. ISSN: 1092-2172.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
ENTRY MONTH: 199812
ENTRY WEEK: 19981203
AB The cyclic AMP (cAMP)-dependent protein kinase, **PKA**, is dispensable for growth of Dictyostelium cells but plays a variety of crucial roles in development. The catalytic subunit of **PKA** is inhibited when associated with its regulatory subunit but is activated when cAMP binds to the regulatory subunit. Deletion of pkaR or overexpression of the gene encoding the catalytic subunit, pkaC, results in constitutive activity. Development is independent of cAMP in strains carrying these genetic alterations and proceeds rapidly to the formation of both spores and stalk cells. However, morphogenesis is aberrant in these mutants. In the wild type, **PKA** activity functions in a circuit that can spontaneously generate pulses of cAMP necessary for long-range aggregation. It is also essential for transcriptional activation of both prespore and prestalk genes during the slug stage. During culmination, **PKA** functions in both prespore and prestalk cells to regulate the relative timing of terminal differentiation. A positive **feedback loop** results in the rapid release of a signal peptide, SDF-2, when prestalk cells are exposed to low levels of SDF-2. The signal transduction pathway that mediates the response to SDF-2 in both prestalk and prespore cells involves the two-component system of DhkA and RegA. When the cAMP phosphodiesterase RegA is inhibited, cAMP accumulates and activates **PKA**, leading to vacuolation of stalk cells and encapsulation of spores. These studies indicate that multiple inputs regulate **PKA** activity to control the relative timing of differentiations in Dictyostelium.

L33 ANSWER 3 OF 9 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1998260039 MEDLINE
DOCUMENT NUMBER: 98260039
TITLE: Coupling gene expression to cAMP signalling: role of CREB and CREM.
AUTHOR: Sassone-Corsi P
CORPORATE SOURCE: Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, C.U. de Strasbourg, France.
SOURCE: INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, (1998 Jan) 30 (1) 27-38. Ref: 57
Journal code: CDK. ISSN: 1357-2725.

PUB. COUNTRY: ENGL[redacted] United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY WEEK: 19980903

AB Several endocrine and neuronal functions are governed by the cAMP-dependent pathway. Transcriptional regulation upon stimulation of this pathway is mediated by a family of cAMP-responsive nuclear factors. This family consists of a large number of members, which may act as activators or repressors. These factors contain the basic domain/leucine zipper motifs and bind as dimers to cAMP-response elements (CRE). CRE-binding protein (CREBs) function is modulated by phosphorylation by several kinases. Direct activation of gene expression by CREB requires phosphorylation by the cAMP-dependent **PKA** to serine 133. Among the repressors, ICER (Inducible cAMP Early Repressor) deserves special mention. ICER is generated from an alternative CREM promoter and is the only inducible CRE-binding protein. ICER negatively autoregulates the alternative promoter, generating a **feedback loop**. ICER expression is tissue specific and developmentally regulated. The kinetics of ICER expression are characteristic of an early response gene. CREM plays a key physiological and developmental role within the hypothalamic-pituitary-gonadal axis. The transcriptional activator CREM is highly expressed in postmeiotic cells. The role of CREM in spermiogenesis was addressed using CREM knock-out mice. Spermatogenesis stops at the first step of spermiogenesis in the mutants and there is a significant increase in apoptotic germ cells. This phenotype is reminiscent of cases of human infertility. ICER is regulated in a circadian manner in the pineal gland, the site of the hormone melatonin production. This night-day oscillation is driven by the endogenous clock (located in the suprachiasmatic nucleus). The synthesis of melatonin is regulated by a rate-limiting enzyme, serotonin N-acetyltransferase (NAT). Analysis of the CREM-null mice and of the promoter of the NAT gene revealed that ICER controls the amplitude and rhythmicity of NAT, and thus the oscillation in the hormonal synthesis of melatonin.

L33 ANSWER 4 OF 9 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 97184174 MEDLINE
DOCUMENT NUMBER: 97184174
TITLE: Protein kinase A (**PKA**)- and protein kinase C-phosphorylated glia maturation factor promotes the catalytic activity of **PKA**.
AUTHOR: Zaheer A; Lim R
CORPORATE SOURCE: Department of Neurology, Division of Neurochemistry and Neurobiology, University of Iowa College of Medicine and Veterans Affairs Medical Center, Iowa City, Iowa 52242, USA.
CONTRACT NUMBER: DK-25295 (NIDDK)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Feb 21) 272 (8) 5183-6.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199706

AB We observed previously that glia maturation factor (GMF), a 17-kDa brain protein, is rapidly phosphorylated in astrocytes following stimulation by phorbol ester, and that protein kinase A (**PKA**)-phosphorylated GMF is a potent inhibitor of extracellular signal-regulated kinase (ERK) and enhancer of p38; both are subfamilies of mitogen-activated protein (MAP) kinase, suggesting GMF as a bifunctional regulator of the MAP kinase

cascades. In the current report, we present evidence that **PKA**-phosphorylated GMF also promotes (11-fold) the catalytic activity of **PKA** itself, resulting in a positive **feedback loop**. Furthermore, GMF phosphorylated by protein kinase C (PKC), but not by casein kinase II or p90 ribosomal S6 kinase, also activates **PKA** (7-fold). It appears that the mutual augmentation of GMF and **PKA**, and the stimulating effect of PKC, both serve to maximize the influence of **PKA** on the regulation of MAP kinase cascades by GMF. Using synthetic peptide fragments containing putative phosphorylation sites of GMF, we demonstrate that **PKA** is capable of phosphorylating threonine 26 and serine 82, whereas PKC, p90 ribosomal S6 kinase, and casein kinase II, can phosphorylate serine 71, threonine 26, and serine 52, respectively. The generation of various phospho-isoforms of GMF may explain its modulation of signal transduction at multiple locations.

L33 ANSWER 5 OF 9 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97334341 MEDLINE
DOCUMENT NUMBER: 97334341
TITLE: Histidine kinases in signal transduction pathways of eukaryotes.
AUTHOR: Loomis W F; Shaulsky G; Wang N
CORPORATE SOURCE: Department of Biology, University of California San Diego, La Jolla 92093, USA.
SOURCE: JOURNAL OF CELL SCIENCE, (1997 May) 110 (Pt 10) 1141-5.
Ref: 26
Journal code: HNK. ISSN: 0021-9533.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY WEEK: 19971003
AB Autophosphorylating histidine kinases are an ancient conserved family of enzymes that are found in eubacteria, archaebacteria and eukaryotes. They are activated by a wide range of extracellular signals and transfer phosphate moieties to aspartates found in response regulators. Recent studies have shown that such two-component signal transduction pathways mediate osmoregulation in Saccharomyces cerevisiae, Dictyostelium discoideum and Neurospora crassa. Moreover, they play pivotal roles in responses of Arabidopsis thaliana to ethylene and cytokinin. A transmembrane histidine kinase encoded by dhkA accumulates when Dictyostelium cells aggregate during development. Activation of DhkA results in the inhibition of its response regulator, RegA, which is a cAMP phosphodiesterase that regulates the cAMP dependent protein kinase **PKA**. When **PKA** is activated late in the differentiation of prespore cells, they encapsulate into spores. There is evidence that this two-component system participates in a **feedback loop** linked to **PKA** in prestalk cells such that the signal to initiate encapsulation is rapidly amplified. Such signal transduction pathways can be expected to be found in a variety of eukaryotic differentiations since they are rapidly reversible and can integrate disparate signals.

L33 ANSWER 6 OF 9 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 97319476 MEDLINE
DOCUMENT NUMBER: 97319476
TITLE: Albumin endocytosis in OK cells: dependence on actin and microtubules and regulation by protein kinases.
AUTHOR: Gekle M; Mildenberger S; Freudinger R; Schwerdt G; Silbernagl S
CORPORATE SOURCE: Department of Physiology, University of Wurzburg, Germany.
SOURCE: AMERICAN JOURNAL OF PHYSIOLOGY, (1997 May) 272 (5 Pt 2)

F668-
Journal code: 3U8. ISSN: 0002-9513.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709

AB We used proximal tubule-derived opossum kidney (OK) cells to determine the dependence of albumin endocytosis on regulation by protein kinases and on the cytoskeleton. Uptake was observed only across the apical but not the basolateral membrane and exceeded uptake in collecting duct-derived Madin-Darby canine kidney cells 14-fold. Inhibition of endocytosis via clathrin-coated vesicles but not via caveolae abolished uptake. Cytochalasin D reduced uptake to < 5% of control, and inhibition of microtubule polymerization by nocodazole reduced uptake to approximately 55% of control. Activation of protein kinase A (**PKA**) by adenosine 3',5'-cyclic monophosphate, forskolin, or parathyroid hormone (PTH) reduced uptake to approximately 65% of control. Protein kinase C (PKC) activation did affect uptake to a similar extent as **PKA** activation but with a certain delay. Stimulation of PKG by guanosine 3',5'-cyclic monophosphate did not affect albumin endocytosis. The inhibitor of tyrosine kinases (TRK), genistein, induced an increase of uptake to approximately 160% of control. Reexocytosis of albumin was enhanced by PKC activation but not by **PKA** activation. TRK inhibition reduced the rate of reexocytosis. We conclude that albumin endocytosis in OK cells requires the integrity of the actin cytoskeleton. Microtubules facilitate endocytosis. Uptake is regulated by **PKA**, PKC, and TRK, yet with different time course and by different mechanisms, e.g., reexocytosis. Possibly TRK activity serves in a negative **feedback loop** to limit albumin endocytosis via a stimulation of reexocytosis.

L33 ANSWER 7 OF 9 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97284020 MEDLINE
DOCUMENT NUMBER: 97284020
TITLE: Prostaglandin E2 stimulates insulin-like growth factor binding protein-4 expression and synthesis in cultured human articular chondrocytes: possible mediation by Ca(++)-calmodulin regulated processes.
AUTHOR: Di Battista J A; Dore S; Morin N; He Y; Pelletier J P; Martel-Pelletier J
CORPORATE SOURCE: Department of Medicine, University of Montreal, (Qc), Canada.. dibattig@ere.umontreal.ca
SOURCE: JOURNAL OF CELLULAR BIOCHEMISTRY, (1997 Jun 1) 65 (3) 408-19.
Journal code: HNF. ISSN: 0730-2312.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY WEEK: 19970904

AB Insulin-like growth factor-1, IGF-1, is believed to be an important anabolic modulator of cartilage metabolism whose action is mediated by high affinity cell surface receptors and bioactivity and bioavailability regulated, in part, by IGF-1 binding proteins (IGFBPs). Prostaglandin E2 (PGE2) stimulates collagen and proteoglycan synthesis in cartilage via an autocrine **feedback loop** involving IGF-1. We determined whether the eicosanoid could regulate IGFBP-4, a major form expressed by chondrocytes and, as such, act as a modifier of IGF-1 action at another level. Using human articular chondrocytes in high-density primary culture, Western and Western ligand blotting to measure secreted IGFBP-4 protein, and Northern analysis to monitor IGFBP-4 mRNA levels, we demonstrated that PGE2 provoked a 2.7 +/- 0.3- and 3.8 +/- 0.5- (n = 3) fold increase in IGFBP-4 mRNA and protein, respectively. This effect was reversed by the Ca(++) channel blocker, verapamil, and the Ca(++)/calmodulin inhibitor,

W-7. The Ca(++)ionophore, ionomycin, mimicked the effects of PGE2. The phorbol ester, PMA, which activated phospholipid-dependent protein kinase C (PKC) in chondrocytes, had no effect on IGFBP-4 production. Cyclic AMP mimetics and **PKA** activators, IBMX, and Sp-cAMP, inhibited the expression of the binding protein as did the PGE2 secretagogue, interleukin-1 beta (IL-beta). The inhibitory effect of the latter cytokine was mediated by a erbstatin/genistein (tyrosine) sensitive kinase. Dexamethasone, an inhibitor of cyclooxygenase (COX-2) expression and PGE2 synthesis, down-regulated control, constitutive levels of IGFBP-4 mRNA and protein, eliminating the previously demonstrated possibility of cross-talk between glucocorticoid receptor (GR) and PGE2-receptor signalling pathways. The results suggest that extracellular signals control IGFBP-4 production by a number of different transducing networks with changes in Ca(++) and calmodulin activity exerting a strong positive influence, possibly maintaining the constitutivity of IGFBP-4 synthesis under basal conditions. PGE2 activation of the IGF-1/IGFBP axis may play a pivotal role in the metabolism of cartilage and possibly connective tissues in general. Eicosanoid biosynthesis may be a rate-limiting step in cartilage repair processes.

L33 ANSWER 8 OF 9 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 96224829 MEDLINE
DOCUMENT NUMBER: 96224829
TITLE: In vitro inhibition of MAP kinase (ERK1/ERK2) activity by phosphorylated glia maturation factor (GMF).
AUTHOR: Zaheer A; Lim R
CORPORATE SOURCE: Department of Neurology (Division of Neurochemistry and Neurobiology), University of Iowa College of Medicine, Iowa City, USA.
CONTRACT NUMBER: DK-25295 (NIDDK)
SOURCE: BIOCHEMISTRY, (1996 May 21) 35 (20) 6283-8.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199609

AB We report that recombinant glia maturation factor (GMF), a 17-kDa brain protein, inhibits the activity of mitogen-activated protein (MAP) kinase in the test tube assay, in particular the ERK1/ERK2 isoforms. A preliminary phosphorylation of GMF by protein kinase A (**PKA**) dramatically increases its inhibitory effect by over 600-fold (Ki approximately 3 nM), making it the most potent MAP kinase inhibitor ever reported. Immunoprecipitation of GMF from cell extracts using its specific antibody coprecipitates ERK (and vice versa), suggesting the association of the two proteins in the cell. The inhibitory effect of **PKA** -phosphorylated GMF is specific, as it does not suppress the activity of cdc2 kinase, another proline-directed kinase. Nor does it inhibit MAP kinase kinase (MEK) and MAP kinase-activated protein (MAPKAP) kinase-2, the two enzymes immediately upstream and downstream, respectively, of ERK. Of the other three enzymes that can phosphorylate GMF, only p90 ribosomal S6 kinase (RSK) enhances the inhibitory function of GMF on ERK; protein kinase C (PKC) and casein kinase II (CKII) are without effect. The inhibition of ERK by **PKA**-phosphorylated GMF suggests that GMF could be one of the mediators of the suppressive effect of the **PKA** pathway on the MAP kinase pathway. On the other hand, that RSK-phosphorylated GMF also inhibits ERK implies a negative **feedback loop** in the regulation of MAP kinase activity.

L33 ANSWER 9 OF 9 MEDLINE
ACCESSION NUMBER: 93363881 MEDLINE
DOCUMENT NUMBER: 93363881
TITLE: Dopamine D2 receptor signaling via the arachidonic acid

cascade modulation by cAMP-dependent protein kinase A and prostaglandin E2.
AUTHOR: Piomelli D; Di Marzo V
CORPORATE SOURCE: Unite de Neurobiologie et Pharmacologie de l'INSERM, Paris,
France.
SOURCE: JOURNAL OF LIPID MEDIATORS, (1993 Mar-Apr) 6 (1-3) 433-43.
Ref: 31
Journal code: A6K. ISSN: 0921-8319.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
AB Recent studies have shown that, in Chinese hamster ovary cells transfected with D2-receptor cDNA, CHO(D2) cells, D2 agonists are potent in enhancing the release of [3H]arachidonic acid (AA) induced by stimulation of constitutive purinergic receptors or by application of Ca2+ ionophores. This facilitatory action is further amplified by the concomitant activation of D1 receptors, which per se have no effect on evoked [3H]AA release. Here, we review a series of experiments aimed at examining the molecular mechanism of this synergistic interaction. The results show that, in CHO(D2) cells: (a) application of 8-Br-cAMP or stimulation of constitutive prostaglandin (PG)E2 receptors augment the AA response produced by D2 agonists; (b) in CHO(D2) cells transfected with human beta 2-receptor cDNA, the beta-agonist, isoproterenol, produces a similar effect; (c) the potentiation of [3H]AA release produced by PGE2 and 8-Br-cAMP is prevented by overexpressing either a protein inhibitor of cAMP-dependent protein kinase (**PKA**) or a mutated form of **pKA** regulatory subunit incapable of binding cAMP; (d) mock-synergism is obtained in CHO(D2) cells overexpressing the catalytic subunit of **PKA**; (e) PGE2 is a major AA metabolite in stimulated CHO(D2) cells and its formation may contribute to the effect of D2 agonists on AA release. The results indicate that cAMP-induced activation of **PKA** represents a likely molecular basis for D1/D2 receptor synergism on AA release. They also suggest that additional membrane receptors, colocalized with D2 and positively linked to adenylyl cyclase, may exert a similar action. Furthermore, stimulation of PGE2 receptors by endogenously produced prostaglandin may participate in AA signaling at the D2 receptor, by providing a paracrine positive **feedback loop**.

=> d his

(FILE 'HOME' ENTERED AT 14:41:49 ON 02 MAY 2000)

FILE 'MEDLINE, BIOSIS' ENTERED AT 14:42:02 ON 02 MAY 2000

L1	128	S	CYCLASE AND CRE
L2	2756	S	(FEEDBACK LOOP)
L3	1	S	L1 AND L2
L4	3561	S	CREB
L5	15	S	L2 AND L4
L6	9	DUP REM	L5 (6 DUPLICATES REMOVED)
L7	35925	S	REPORTER
L8	2	S	L6 AND L7
L9	4	S	AUTOPOSITIVE
L10	30	S	AUTO-FEEDBACK
L11	77963	S	AMPLIFICATION
L12	131206	S	AMPLIF?
L13	276	S	REPORTER ASSAY
L14	2788	S	L2 OR L9 OR L10
L15	0	S	L13 AND L14
L16	47	S	L14 AND REPORTER

L17	28	DUP REM L[illegible] (19 DUPLICATES REMOVED)
L18	28	S L14 AND L17
L19	3	S L17 AND L12
L20	3124	S REPORTER CONSTRUCT?
L21	77963	S L11
L22	77	S L11 AND L20
L23	4	S L22 AND L14
L24	2	DUP REM L23 (2 DUPLICATES REMOVED)
L25	1	S L1 AND L12
L26	37	S L1 AND L7
L27	0	S L26 AND L16
L28	0	S L26 AND L14
L29	20	DUP REM L26 (17 DUPLICATES REMOVED)
L30	13729	S PKA
L31	16	S L30 AND L14
L32	16	S L30 AND L14
L33	9	DUP REM L32 (7 DUPLICATES REMOVED)
L34	0	S L33 AND L7

mic

STIC-ILL

From: Brannock, Michael
Sent: Tuesday, May 02, 2000 3:39 PM
To: STIC-ILL
Subject: 09/378046

Q1. N2

Please provide the following refs:

Sassone-Corsi, P. International Journal of Biochemistry and Cell Biology 30(1)(27-38)1998

Peunova, N. et al Nature 364 no. 6436 pp 450-453 1993

Fur, G et al European Journal of Pharmacology 374(3)445-55, 1999

thank you

Michael T. Brannock, Ph.D.
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most areas of adult brain[16,19], suggesting that they may contribute to cellular and synaptic differentiation in the developing central nervous system. Thus, the deficit of $\alpha 5$, $\beta 3$ and $\gamma 3$ subunits is likely to cause deleterious effects such as the tremor and jerky gait that appear in p^{cp} mice. Moreover, it is possible that a $\beta 3$ deficit leads to other development anomalies associated with p^{cp} (cleft palate and runting for example), because $\beta 3$ is expressed in germinal zones[16] and the mitotic zone of the forebrain[22]. Alternatively, the absence of other, as-yet unidentified, gene(s) in the p^{cp} deletion may be responsible for these phenotypes. Analysis of temporal and spatial expression patterns of $GABA_A$ receptor subunit, together with histological analysis of the developing p^{cp} brain, could provide an insight into the neurological and cellular basis of p^{cp} phenotypes, and also clarify the significance of $\alpha 5$, $\beta 3$ and $\gamma 3$ subunits in development.

The human counterpart of the region deleted in p^{cp} is associated with Angleman syndrome (AS)[5-9], which is characterized by severe mental retardation, microcephaly, seizures, ataxia, craniofacial anomalies and hypopigmentation[23,24]. The smallest known maternal deletion resulting in AS involves the $\beta 3$ but not the $\alpha 5$ gene[8,9] (although a single AS patient bearing a translocation is apparently intact for the $\beta 3$ gene[25]). Because the phenotypic effects of the p^{cp} mutation are recessive and independent of parental origin[2], the AS critical region may be outside of the p^{cp} deletion, despite certain phenotypic similarities between p^{cp} mice and AS patients[15]. However, if the mouse counterpart of the AS gene(s) is not imprinted, it is possible that the AS critical gene(s) may be within the p^{cp} deletion. Indeed, an AS-like paternal imprinting effect was not detected for the central region of mouse chromosome 7 (including the region deleted in the p^{cp} mutation[26]). Conservation of synteny of the region deleted in p^{cp} with human chromosome 15q predicts that the human $\gamma 3$ gene will map near the $\alpha 5$ and $\beta 3$ genes. It thus remains to be determined what role, if any, these three $GABA_A$ receptor subunit genes play in the aetiology of AS.

Note added in proof: Culiat *et al.*[32] have reported a concordance of $\beta 3$ gene disruption and cleft palate phenotype in a series of *p* allele deletions. □

Received 12 March; accepted 21 May 1993.

1. Silvers, W. K. *The Coat Colors of Mice* 90–101 (Springer, New York, 1979).
2. Lyon, M. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **89**, 6968–6972 (1992).
3. Phillips, R. J. S. *Mouse Newsletter* **56**, 18 (1977).
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Amplification of calcium-induced gene transcription by nitric oxide in neuronal cells

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NITRIC oxide (NO) is a short-lived, highly reactive gas, which has been identified as a mediator in vasodilation, an active agent in macrophage cytotoxicity and neurotoxicity, and a neurotransmitter in the central and peripheral nervous systems[1-5]. Production of NO by neurons is critical for facilitated synaptic transmission in models of synaptic plasticity such as long-term potentiation and long-term depression, suggesting a role for NO as a retrograde messenger that could complete a hypothetical feedback loop by strengthening the connection between postsynaptic and presynaptic cells[6-10]. We report here that although alone NO has no evident effect on transcription, it can act as an amplifier of calcium signals in neuronal cells. NO and Ca^{2+} action have to coincide in time for amplification to occur. Experiments with a series of simplified reporter genes in combination with specific recombinant protein kinase inhibitors suggest that induction of gene activity following NO-amplified calcium action involves protein kinase A-dependent activation of the transcription factor CREB.

To model a situation in which a neuron receives a NO signal simultaneously with a signal of a different modality, we exposed neuronal PC12 cells to combinations of NO with different inducers of transcription and monitored changes in c-*fos* and c-*jun* messenger RNAs. These immediate-early genes serve as sensitive reporters[11], the modular structure of their promoter regions allowing correlation of incoming signals with discrete transcriptional regulatory elements and factors. Further, these genes encode components of a family of transcription factors that may mediate subsequent long-term changes in patterns of cellular gene expression[11]. Figure 1*a* shows that NO (produced by a nitric oxide-generating compound, sodium nitroprusside, SNP) was unable to induce transcription on its own; this holds true over a wide range (from 0.1 μM to 1 mM) of SNP concentrations (data not shown). But when NO was applied to cells in combination with certain treatments known to induce immediate-early gene expression, induction of c-*fos* expression was greatly enhanced in some cases. NO only amplified the action of those agents that act through calcium ions — ionophores A23187 and ionomycin, Bay K8644 (an agonist of voltage-gated calcium channels (VGCC), thapsigargin (an agonist of intracellular calcium release) and KCl, which depolarizes the cell membranes. In contrast, NO did not affect induction by forskolin or phorbol myristate acetate (PMA). NO had similar effects on c-*jun* induction, although to a much lesser degree. NO-mediated amplification was blocked by the Ca^{2+} chelator EGTA, by the VGCC

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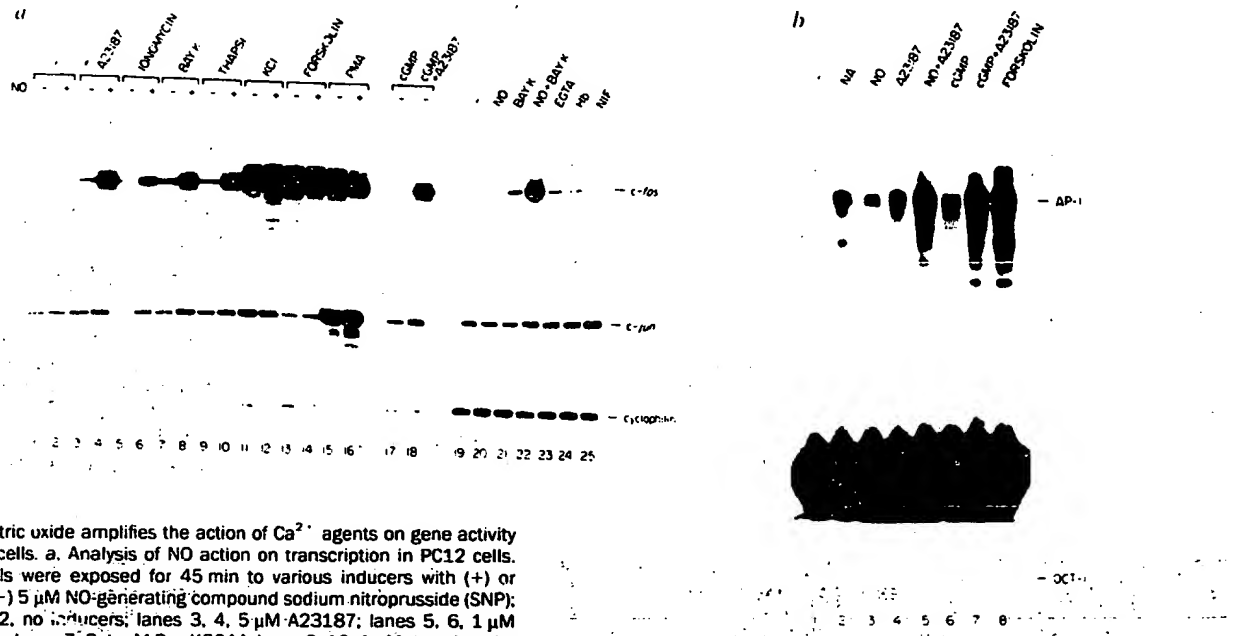

FIG. 1 Nitric oxide amplifies the action of Ca^{2+} agents on gene activity in PC12 cells. *a*, Analysis of NO action on transcription in PC12 cells. PC12 cells were exposed for 45 min to various inducers with (+) or without (−) 5 μM NO-generating compound sodium nitroprusside (SNP); lanes 1, 2, no inducers; lanes 3, 4, 5 μM A23187; lanes 5, 6, 1 μM ionomycin; lanes 7, 8, 5 μM Bay K8644; lanes 9, 10, 1 μM thapsigargin; lanes 11, 12, 55 mM KCl; lanes 13, 14 10 μM forskolin and 0.5 mM isobutylmethylxanthine (IBMX); lanes 15, 16, 200 ng ml^{-1} PMA; lane 17, 20 μM 8-Br-cGMP; lane 18, 20 μM 8-Br-cGMP and 5 μM A23187. Lanes 19–25 represent a separate set of experiments where the cells received no treatment (lane 19), 5 μM SNP (lane 20), 5 μM Bay K8644 (lane 21) or a combination of both (lanes 22–25), but were also treated with 3 mM EGTA (lane 23), 500 μg ml^{-1} of haemoglobin (lane 24) and 20 μM nifedipin (lane 25) 5 min before addition of SNP and Bay K8644. *b*, Enhancement of AP-1 binding activity by combined NO/Ca^{2+} action. Nuclear extracts were prepared from untreated cells (lane 2) and from cells treated with 5 μM SNP (lane 3), 5 μM A23187 (lane 4), a combination of both (lane 5), 20 μM 8-Br-cGMP without (lane 6) or with (lane 7) 5 μM A23187, or 10 μM forskolin and 0.5 mM IBMX (lane 8). Nuclear extracts were incubated with ^{32}P-labelled probes corresponding to the AP-1 binding site (AP-1) or the octamer motif (Oct-1) from the HSV ICP0 promoter.

METHODS. PC12 cells were grown in DMEM supplemented with 5% calf serum and 10% horse serum (HyClone). For induction, cells were exposed for 45 min to various inducers in the presence or absence of 5 μM SNP, dissolved in H_2O immediately before addition. Cells were collected on ice, washed with ice-cold PBS and the cytoplasmic RNA was extracted and analysed by RNase protection following standard procedures. The c-*fos* probe was prepared from a plasmid carrying mouse c-*fos* sequences from −56 to +109. The c-*jun* probe contained rat c-*jun* sequences from +295 to +527; cyclophilin probe (a gift from G. D'Arcangelo) contained rat cyclophilin sequences from +465 to +673. Rat c-*fos* mRNA carries three mismatches with the mouse probe, but under our T1 RNase digestion conditions it protected an RNA fragment of the expected length. The enhancement effect was reproduced by different sources of NO, including S-nitrosocysteine and 3-morpholino-sydnonimine (data not shown), suggesting that this effect is specific to NO. For band-shift analysis, PC12 cells were stimulated for 90 min, washed with ice-cold PBS and the nuclei were isolated by treatment with 0.1% NP-40. Nuclear proteins were extracted in high-salt buffer and binding assays were as described[14]. Synthetic oligonucleotides contained a single copy of AP-1 site (5'-TCGACGGTATCGATAAGCTATGACTCATCCGGGGGATC-3') (a gift from K. Riabowol). Experiments with unlabelled wild-type and mutant AP-1 binding oligonucleotides and Fos- and Jun-specific antibodies confirmed that this elevation was due to specific binding by Fos- and Jun-containing protein complexes (data not shown). Treatment of PC12 cells with forskolin and IBMX produced the biggest increase in AP-1 binding activity and is reproduced (*b*, lane 8) for comparison with other inducers. For Oct-1 assays the same conditions were used except that each reaction contained 0.1 vol. of fetal bovine serum and the probe used was a 120 bp fragment containing a TAATGARAT element derived from the HSV ICP0 promoter (a gift from J. S. Lai).

antagonist nifedipin, and by extracellular haemoglobin, which binds NO and sequesters it from the media (lanes 23–25). The NO/Ca^{2+} induction of the endogenous c-*fos* gene and a transfected *fos* promoter showed rapid and transient kinetics characteristic of c-*fos* activation by a variety of inducers[11,12] (Fig. 2*a*).

The elevated induction of c-*fos* mRNA was productive, leading to an increase in binding activity of the AP-1 transcription factor comprising Fos and Jun family proteins. The combined action of NO and the Ca^{2+} ionophore A23187 produced a strong increase in AP-1 activity (Fig. 1*b*).

To test the possibility that NO acted by changing the level of cellular cyclic GMP[13], we treated the cells with a combination of A23187 and 8-Br-cGMP. Application of 8-Br-cGMP did not induce transcription, but, like NO, 8-Br-cGMP amplified the effect of Ca^{2+} influx (Fig. 1*a*, lanes 17, 18). This effect was also reproduced when AP-1 binding was measured (Fig. 1*b*). These data support the notion that cGMP may mediate NO action and indicate that cGMP can influence gene expression. Taken together, these results suggest that the combined action of NO and calcium leads to production of a highly active transcription factor capable of reprogramming the pattern of gene expression in neurons.

To determine whether NO and calcium act coordinately to produce a synergistic effect on transcription, we gave a pulse of one component, washed it away and added the second component immediately or after a further incubation of 5, 10 or 20 min. Addition of Bay K8644 after the removal of NO resulted in a loss of the synergistic effect (Fig. 2*b*, lanes 9–12). This was observed even when application of the two inducers was separated by only 5 min or less. After 10 min the signal was back to the levels of induction by Bay K8644 alone (compare lanes 3, 11 and 12). In the reciprocal experiment, in which Bay K8644 was applied first, a similar loss in the synergistic action of the two signals was observed (lanes 5–8). As a control, if the second reagent was added after 10 min without removing the first, the amplified response was nearly unaffected (lanes 13, 14). These

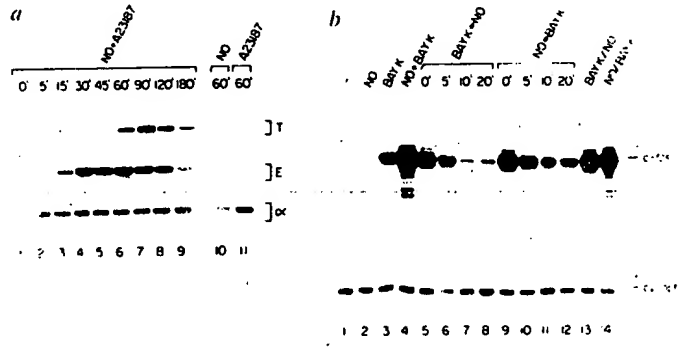

FIG. 2 Time requirements for the synergistic NO/Ca^{2+} action. *a*, Kinetics of induction of endogenous and transfected *fos* genes after NO/Ca^{2+} action. Reporter constructs containing CAT gene fused to the −356–+109 promoter region of c-*fos* gene (−356-*fos*-CAT) and a human α-globin gene used as an internal control were transfected into PC12 cells. After induction by 5 μM SNP and 5 μM A23187, RNA was isolated at various time points, indicated on the figure, and analysed by RNase protection assay as described for Fig. 1. T, E and α mark the positions of probe fragments protected by correctly initiated transcripts of the transfected c-*fos*-CAT plasmid, endogenous c-*fos* RNA and transcripts of the α-globin internal control, respectively. *b*, Analysis of the time window of the synergistic NO/Ca^{2+} action. PC12 cells were left untreated (lane 1), or were exposed to 5 μM SNP, 5 μM Bay K8644, or a mixture of SNP and Bay K8644 (lanes 2, 3 and 4, respectively). In a separate series of experiments (lanes 5–8) PC12 cells received a pulse of 5 μM SNP for 10 min, were washed several times with warm equilibrated DMEM, covered with warm equilibrated DMEM and, after indicated periods of time, received 5 μM Bay K8644 for 45 min; in reciprocal experiments (lanes 9–12) cells received a 10 min pulse of 5 μM Bay K8644, followed by washing, incubation and addition of 5 μM SNP after indicated periods of time. Lane 13, Bay K8644 (5 μM) was added to cells for 10 min followed by addition of 5 μM SNP for 45 min without removal of Bay K8644; lane 14, SNP (5 μM) was added to cells followed by addition of 5 μM Bay K8644 without removal of SNP. RNA was isolated and assayed for c-*fos* RNA as described for Fig. 1.

METHODS. Plasmids were transfected into PC12 cells by electroporation at 220 V, 960 μF. After a 40-h incubation the cells were stimulated for the indicated period of time, collected, and RNA was isolated as described in Fig. 1, except that RNA preparations were treated with RNase-free DNAase and purified by deproteinization. RNA was analysed by RNase protection assay using c-*fos* and α-globin-specific probes as described for Fig. 1. The α-globin probe contained human α-globin gene sequences from −15 to +95.

observations indicate that the two stimuli, NO and calcium, have to be present together within a small time window to exert their synergistic action.

Calcium induction of a transfected reporter gene composed of the c-*fos* promoter linked to the heterologous chloramphenicol acetyltransferase coding sequences (c-*fos*-CAT)[13,14] can also be amplified by NO, indicating that a short (400-base-pair (bp)) promoter region of the c-*fos* gene is sufficient to confer NO/Ca^{2+} inducibility (Fig. 2*a*). To examine which DNA sequences in the c-*fos* promoter and which transcription factors mediate this effect, we tested a series of simplified reporter constructs[13,14] (a gift from M. Gilman) for their ability to support NO-amplified calcium signalling. Promoter deletion analysis (Fig. 3*b*) shows two major transition points in induction levels, first when the reporter promoter was truncated from −356 to −275, which removed several important regulatory elements, and secondly when it was truncated from −71 to −56, removing the major cAMP-responsive element (CRE). These results are consistent

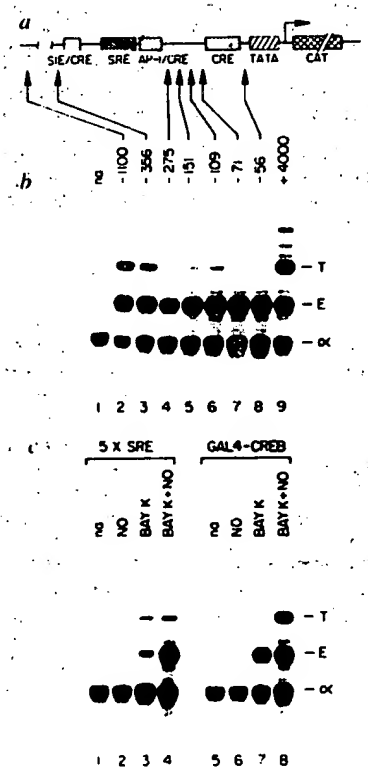

FIG. 3 CRE/CREB can transmit the amplified NO/Ca^{2+} signal to a reporter gene. *a*, Schematic diagram of c-*fos* upstream sequences. The regions corresponding to the *sis*-inducible element (SIE/CRE), the serum response element (SRE), the AP-1 binding site (AP-1/CRE), the cAMP response element (CRE), TATA box (TATA) and the CAT reporter gene are shown as filled boxes. *b*, Transient expression assay of mouse c-*fos* 5′-deletions. A series of reporter constructs containing truncated mouse c-*fos* promoter carrying sequences up to +109 and connected to the coding region of CAT gene[14] were transfected into PC12 cells along with the human α-globin internal control plasmid. Numbers and arrows above the lanes show the positions of 5′-end points of deletions of the c-*fos* promoter in c-*fos*-CAT fusions; lane 9 contained c-*fos* gene from position −356 to +4,000. *c*, Transient expression assay of multimerized SRE and of the GAL4/CREB reporter system. Cells were transfected with a reporter construct containing five copies of SRE connected to position −56 of c-*fos* promoter of the c-*fos*-CAT construct (5 × SRE, lanes 1–4), or with a mixture of 3 μg of reporter plasmid 5 × GAL4-CAT and 0.3 μg of expressor plasmid GAL4-CREB (GAL4-CREB, lanes 5–8) or its mutated (Ser 133→Ala) version (lanes 9–12). The constructs were the same as in ref. 20, except that GAL4-CREB fusion was expressed under the control of RSV promoter. All transfections contained α-globin internal control plasmid. Cells were transfected and RNA was assayed as described in Fig. 2. Series of truncated c-*fos*-promoter, 5 × SRE-CAT, 5 × GAL4-CAT and GAL4-CREB constructs were gifts from M. Gilman and L. Berkowitz.

with the function of the c-*fos* CRE as one, but not the sole, Ca^{2+} response element (refs 15, 16 and N.P., M. Gilman and G.E., manuscript in preparation). To test whether the c-*fos* serum response element (SRE), which can also transmit part of a Ca^{2+}-generated signal in KCl-depolarized PC12 cells (N.P. *et al.*, manuscript in preparation), is a target for the NO/Ca^{2+} pathway, we transfected cells with a reporter construct containing five copies of the SRE linked to a truncated c-*fos* promoter devoid of inducible sequences. Data in Fig. 3*c* (lanes 1–4) demonstrate that NO failed to amplify the calcium-dependent activation of this reporter. Thus, the SRE is not a target for NO-amplified Ca^{2+} signals.

CREs of several genes are bound by the transcription factor CREB[17–20]. To test directly the involvement of CREB as a downstream target of NO potentiation and to discriminate CREB-mediated effects from the action of endogenous transcriptional factors, we used effector proteins in which the DNA-binding specificity of CREB has been changed by fusion to the DNA-binding domain of the yeast transcriptional activator GAL4[20]. A reporter gene containing multiple GAL4 binding sites served as a sensitive indicator of the transcriptional activity of the hybrid CREB protein. The combined action of NO and Bay K8644 synergistically activated the reporter gene (Fig. 3*c*). A mutant form of GAL4-CREB lacking the Ser 133 phosphoacceptor site could no longer transmit the NO signal, thus demonstrating that a functional CREB protein is a target for at least one of the NO/Ca^{2+}-induced pathways in the nucleus.

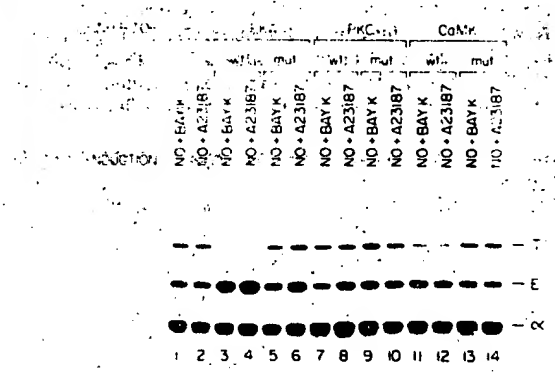

FIG. 4 Analysis of input of different protein kinases in NO-amplified calcium action. PC12 cells were cotransfected with −356-*fos*-CAT reporter construct along with 20 μg of recombinant inhibitors of different protein kinases and human α-globin control. Cells were exposed for 60 min to the action of 5 μM SNP and 5 μM A23187, or 5 μM SNP and 5 μM Bay K8644, as indicated, and RNA was isolated and assayed as described on Fig. 2. Reporter constructs were cotransfected either with pUC119 (lanes 1, 2) or with active (wt) and mutated inactive (mut) versions of recombinant protein kinase inhibitors as indicated. Lanes 3–6, recombinant inhibitors for protein kinase A (PKA); lanes 7–10, inhibitors for protein kinase C (PKC); lanes 11–14, inhibitors for Ca^{2+}/calmodulin-dependent multifunctional protein kinase II (CaMK). Recombinant inhibitors of protein kinases were expressed under the control of RSV promoter and rabbit β-globin splicing/polyadenylation sites. The structures of the recombinant PKC and CaMK inhibitors were matched to the sequences of the short conserved peptides located at positions 19–36 in various forms of PKC and positions 273–302 of CaMK, respectively. The recombinant PKA inhibitors included sequences of the murine PKA regulatory subunit RIα and the natural heat-stable PKA inhibitor (PKI). Such peptides act as powerful and highly specific pseudosubstrate inhibitors of the cognate enzymes *in vitro*[24–26]. Mutant forms of the inhibitors carrying inactivating amino-acid substitutions in the kinase recognition sequences served as controls. In a series of control experiments (N.P. et *al.*, manuscript in preparation and data not shown) the actions of recombinant inhibitors was shown to be selective for their target kinases and potent enough to block the reporter induction by a corresponding stimuli (forskolin, TPA, KCl).

Ca^{2+} signalling in neurons is mediated by several serine/threonine-specific protein kinases[21–23]. To determine which enzymes are involved in signalling by NO/Ca^{2+}, we have used specific recombinant inhibitors of individual protein kinases (N.P. *et al.*, manuscript in preparation). These inhibitors were constructed based on the autoinhibitory pseudosubstrate domains of several protein kinases[24–26] and they are potent and selective toward their cognate enzymes (N.P. *et al.*, manuscript in preparation). We cotransfected reporter plasmids carrying a full-length c-*fos* promoter with inhibitor plasmids and monitored whether selective inhibition of a particular protein kinase blocked NO/Ca^{2+} induction. Mutant inactive forms of the inhibitors served as controls. Consistent with the data on promoter mapping, only the cAMP-dependent protein kinase (PKA) inhibitor and, to a small degree, Ca^{2+}/calmodulin-dependent protein kinase II (CaMK) inhibitor blocked the NO/Ca^{2+} response (Fig. 4). These results implicate the PKA-CREB-CRE system as a major component of the signalling pathway for the transcriptional synergy of NO and Ca^{2+} and suggest that cAMP-Ca^{2+} synergism[15,27] may be a part of NO/Ca^{2+} signalling.

The phenomenon of NO-mediated potentiation of the Ca^{2+} response may have implications beyond transcriptional regulation. For example, NO could potentiate the PKA phosphorylation of cytoplasmic proteins with direct roles in synaptic function. The effect of NO on signalling might be particularly important at very low levels of calcium action, at which this inducer acting alone would have negligible effect; these very weak signals, which would go unnoticed by the cell, might be amplified by NO, resulting in pronounced physiological changes for the cell. Because NO diffuses freely, nearby synapses that receive very weak impulses simultaneously with exposure to NO might establish facilitated synaptic transmission[28]. NO and Ca^{2+} have to act within a very narrow time window for this enhancement to occur, suggesting that in the nervous system, this synergistic effect might be restricted to the recently active synapses, thereby coinciding with transient elevations of calcium levels[29]. □

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